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## A NEW DISEASE IN MOOSE. I.

PRELIMINARY REPORT \*

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During the past ten summers, Dr. Cahn has spent many months in the wilderness of the Superior National Forest of Minnesota, the Quetico Provincial Park adjacent to it in Ontario, Canada, and in the vast territory lying still farther north than these areas. The object of his travels was a study of the moose (*Alces americana americana*) in an effort to obtain a first-hand knowledge of its life history and ecology. From woodsmen, trappers, rangers, hunters he heard repeated over and over again tales of a mysterious disease which causes death in the late winter and early spring of a considerable number of these large animals. Always the symptoms were the same; hence, assuming that there was ground for the reports, he undertook the initial stages of the investigation of this disease. Very quickly the problem ceased to be primarily one of vertebrate zoology, and Dr. Thomas joined the investigation because of its parasitological aspects. Unquestionably the organism with which the writers are dealing is a difficult and obscure one. However, certain definite facts have been ascertained, and the writers offer the results of their investigations as they appear at this time, while further studies are being carried on. They wish to express their appreciation to Mr. W. A. Hanson, district chief warden of the region involving the Superior National Forest of Minnesota, who supplied the ticks from this region, and whose cooperation made the investigation at a distance of nearly a thousand miles from the seat of the disease possible; to the department of Animal Husbandry at the University of Illinois through whose cooperation experimental animals were made available; to Dr. Richard R. Kudo, whose observations and suggestions have facilitated the work; and to Dr. F. W. Tanner of the department of Bacteriology for aid and suggestions regarding the bacteriological aspects of the problem.

### THE MOOSE DISEASE

The disease under discussion attacks the moose of northern Minnesota only during the late winter and early spring, usually between

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\* Contribution from the Zoological Laboratory of the University of Illinois, No. 425.

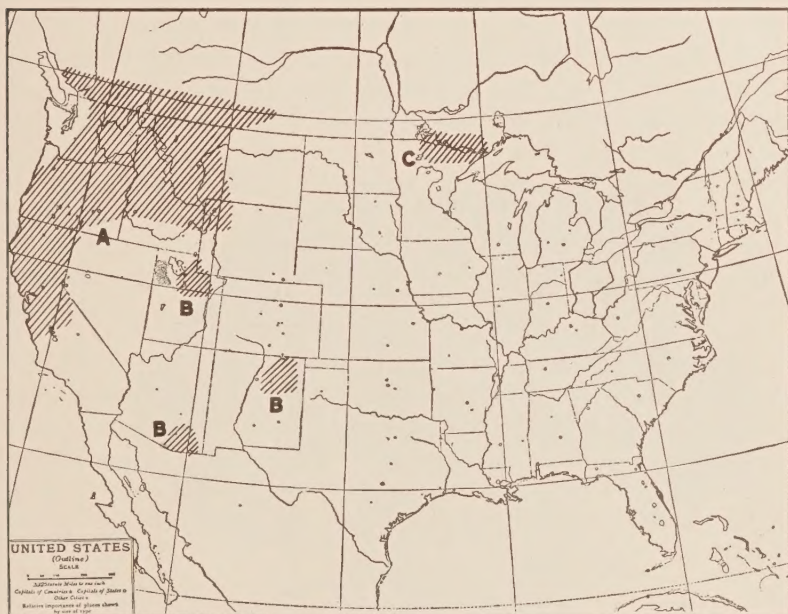
February and May. During this period many moose are discovered covered with ticks (*Dermacentor albipictis* Packard); some of these tick-infested animals are nearly devoid of hair as a result of the ravages of the ectoparasites. Some of them behave strangely: they become extremely active, are constantly on the run; they race blindly through the woods bumping into trees, falling over obstacles, or they take to the open spaces of the lakes. If there be ice, they wander in circles, aimlessly, blindly; if there be open water, they swim about without rime or reason, growing weaker and weaker until they drown. If they do not meet a watery grave, they eventually exhibit a form of paralysis which causes them to break down in the hind quarters, sometimes in the fore quarters. In this helpless condition they struggle to drag themselves about until exhausted, or until a pack of wolves puts an end to their misery. The following brief reports are typical of many at hand: "The first case I recall was at Eagles Nest lake in June, 1926. The moose had been out in the water swimming for at least three hours and when I arrived it was about exhausted. I got in a canoe and drove it out of the lake and into the woods. From all indications the moose appeared to be blind. It would run around in circles, fall down, struggle up as if partially paralyzed and run into trees. I finally had to kill it." "Another moose was at Twin lakes in February. It had been feeding blindly in an area of two or three acres for some time, according to the signs. In running he would hold his head to one side, fall down, stagger up, and crash into trees. It eventually became exhausted and had to be killed." "Burntside Lake in February: the moose was on an island and from indications had been there for a couple of months, for it had eaten practically everything eatable and uneatable on the island. It was staggering, partially paralyzed and in a very weakened condition." "May: a moose captured between Ely and Winton, Minnesota. It was caught in a wire fence and was in such a weakened condition it could not get out. I roped it and took it to a barn, where it died two days later. This was the worst case of tick infestation I have seen: there was practically no hair left on the animal." These are merely specimen cases as reported by Mr. Hanson, and many similar reports are at hand from other observers. All agree in the following symptoms: (1) tick infestation; (2) blind, aimless wandering, staggering, bumping blindly into perfectly obvious obstacles, weakened condition; (3) paralysis (see figures 1, 2).

It seemed to the writers at once that the tick probably was at the bottom of the trouble, not as a primary causative agent but as a transmitter of the disease. The fact that it is a *Dermacentor* was in itself highly suspicious, for nearly every representative of this genus has been shown capable of transmitting diseases such as Rocky Mountain



spotted fever, tick paralysis, and others. In fact, *Dermacentor albipictis* alone has, until recently, remained free of the stigma of disease transmission. Parker (1929) showed that Rocky Mountain spotted fever might be transmitted through the adult tick of this species. Our investigations emphasize further that it can no longer enjoy this freedom: it transmits the organism causing the disease in moose.

*Dermacentor albipictis* is not a native species in Minnesota or in adjacent Ontario (Text fig. A). It is the abundant tick in British Columbia and Saskatchewan, on the western slope of the northern



Text Fig. A.—Map of the distribution of the tick *Dermacentor albipictis*. A: native range. B: occurrences supposedly to transportation on animals. C: region of the Minnesota infection.

portion of the Rocky mountains in the United States, Washington, Oregon, northern California, and in Montana and Idaho. In these regions it is reported as a pest (not a disease carrier) upon cattle, horses, mountain goats, mule, deer, elk, beaver (1 record), moose and domestic rabbits (Bishopp and Wood, 1913). Scattered records exist for Texas, Utah and Arizona, but it is generally supposed that these instances are due to introduction on transported cattle, horses or game. The first report of this tick in Minnesota is that of Howard (1917) who believes that they were imported to the region on elk brought from Montana to stock the Minnesota game refuge two or three years

previously. These elk were so badly infested with *D. albipictis* that spraying was resorted to the *next year*. This idea of an introduced species is concurred in by Riley (1922) who notes that the tick is suspected of causing the death of moose in Minnesota. At the present time the tick has spread in northern Minnesota to deer, wolves, coyote, bear, red fox and snow-shoe rabbit. Infected moose are known from southern Ontario, but as yet there is no indication that the ticks have spread far to the north in this region. The distribution of *D. albipictis* is shown in Text figure A.

#### LIFE HISTORY OF *DERMACENTOR ALBIPICTIS*

The life history of this tick has been worked out in detail by Bishopp and Wood (1913), and may be summarized as follows: The engorged adult, fertilized females drop to the ground in the early spring and lay 1500 to 4500 eggs (Text fig. B). These eggs, after an incubation period of 33-71 days in which temperature and humidity play an important part, hatch into tiny larvae which, after a period of inactivity, regain the host and attach themselves at once. On the host the metamorphosis occurs, the cycle in males being shorter than in females, and after fertilization the engorged adult female drops to the ground and oviposits. One fact the present writers would add to this history: In a piece of moose hide with a heavy tick infestation, received at Urbana, Illinois in late April, the engorged females were found laying their eggs in the tangled moose hair about to be shed. If this be a normal method of procedure, then we have a far more efficient method of tick dissemination, for the moose, wandering through the brush, will leave the eggs widely scattered over the country normally traversed by this animal.

#### EXPERIMENTAL WORK

From the myriad of problems that presented themselves, the writers have fixed their attention on but two: (1) to determine the causative organism producing the disease; and (2) to ascertain the role played by the tick *Dermacentor albipictis* in the transmission of the disease. To bridge the gap of nearly a thousand miles separating the writers from the area of infection, pieces of moose hide cut from infected moose in the Superior National Forest of Minnesota were shipped in sealed containers to the laboratory at Urbana. Here the ticks were removed as needed and kept in jars with moist sand on the bottom. For experimental infestation guinea pigs, rabbits, a bull calf and a lamb were used. In order to prevent the animals from scratching off the ticks and eating them, the hair was clipped from an area in the middle of the back, and the ticks protected by a bandage over the spot





Text Fig. B.—The winter or moose tick, *Dermacentor albipictis*. *a*, adult male; *b*, small nymphal female; *c*, partially engorged female; *d*, adult, engorged female; *e*, adult female *Dermacentor albipictis* ovipositing.

(Figs. 3, 4). This was snugly fitted so as to prevent the escape of the ticks, but not tight enough to inconvenience the animal, and proved highly satisfactory. Careful and complete notes were kept on behavior and reactions of infected animals, and on their death an autopsy was performed. Blood smears of the liver, spleen, kidney, heart, lungs and brain were made, and portions of these organs preserved in Bouin for sectional study. Wright and Giemsa stains were used on the smears for microscopic study. Since guinea pigs proved the most satisfactory, attention was concentrated on them. Eventually pigs were infected with ticks sorted as to sex and stage of development so as to determine whether both sexes and various nymphal stages were capable of transmitting the disease. The following is a summary of the various series of experiments, with their component cases.

SERIES A. Two adult guinea-pigs infested with twenty ticks each, consisting of adult males and nymphal females, on March 18, 1932. At this time there were no adult female ticks available.

- A1. 3/21. Temperature (rectal), 102°F. Pig restless; eyes sunken; breathing short, rapid.
- 3/27. Diarrhea severe; body covered with festering sores marking the spot of each tick attack; hind legs paralyzed, but pig struggling to rise.
- 3/28. Pig died at 8 a. m. in spasms.
- Autopsy: 3 male and 1 nymphal female tick recovered, still attached. Lungs congested; liver congested and spleen almost bloodless; liver with yellow pus-like spots; spleen abnormally small; heart congested.
- A2. 3/27. Diarrhea severe; pig very active; temperature 102.6°F.
- 3/28. Staggering; runs in blind circles, bumping into walls of cage; hind legs with increasing paralysis; finally collapsed. Violent convulsions; given up for dead. After 20 minutes slowly revived and struggled to rise. Almost normal by evening, but weak.
- 3/29. Apparently entirely recovered. Examination of body showed all ticks scratched off.
- 4/3. After being normal, is rather quiet today; breathing short and labored; temperature 102.2°F.
- 4/4. Normal again today.
- 4/24. Notably thin, anemic. Growing drowsy toward afternoon.
- 4/25. Has not moved all day, sitting humped up; neither eating nor drinking; eyes sunken. No paralysis.
- 4/26. Can not raise head; has not moved in two days.
- 8:00 a. m. Respiration, 78; temperature, 102.6°F.
- 3:30 p. m. Respiration, 126; temperature, 103°F.; very weak.
- 5:00 p. m. Respiration down to 102, heavy, labored; hind legs completely paralyzed; do not respond to any stimulus. Temperature, 102.8°F.
- 5:48 p. m. Convulsions and spasms, followed by death.
- Autopsy: Liver yellow, finely mottled; spleen small, almost bloodless; no lesions of any kind evident; lungs normal; kidneys normal; heart engorged.

SERIES B. Two rabbits two months old, infested with 15 adult male and nymphal female ticks on March 22. One rabbit scratched the ticks off during the first night, and has shown no effects to date.

B1. 4/1. Very sluggish today; not eating or drinking.

4/2. Drooling at mouth; paralysis in legs; eyes sunken, lack luster. Struggling to rise.

12 noon. Convulsions and death; legs paralyzed.

Autopsy: 1 male tick recovered. Lungs congested; kidneys dark red, congested; spleen abnormally small, bloodless; festering sores with greenish cores around dorsal rump where ticks have worked.

SERIES C. Three rabbits infested with adult male and nymphal female ticks (ten ticks each) on March 26. They rid themselves of the ticks within 24 hours. No results to date (5/6); series discontinued.

SERIES D. Ayrshire bull, 8 months old. About 100 assorted ticks of both sexes and all developmental stages placed on scrotum and encased in bolting cloth sack on April 23.

4/26. Examined bull and found most of ticks attached, but added a new supply of 50 ticks for safety's sake.

5/3. Bull very uneasy and rather sluggish; temperature, 102.2°F.

5/6. Bull very uneasy, restless; temperature, 102.4°F.

Experiment continued.

SERIES E. Two rabbits given intravenous injection of mashed, engorged adult female ticks in Ringer solution strained through finest bolting cloth; this on April 4. 1 cc. injected into vein in ear.

5/6. No effects noted to date. Series continued.

SERIES F. Heart puncture made in guinea pig A2 on 4/20. 1 cc. blood withdrawn into 1 cc. Ringer solution plus sodium acetate. This (2 cc.) was injected by hypodermic needle directly into the heart of 3 young guinea pigs. Object: to determine if the virus from an infected guinea pig will effect other pigs.

5/6. No effect to date. Series continued.

SERIES G. 23 guinea pigs infested with *Dermacentor albipictis* according to sex and developmental stages on 4/24.

Set G1. 4 pigs infested with 20 adult male ticks each.

G1.1. 4/28. O. K. at 7:30 this morning. At 11:30 showed signs of inactivity: lazy, bunched up.

3:15 p. m. Found lying on back, in extreme spasms; hardly able to get enough blood from ear for smear; weak, periodic spasms followed; all four legs periodically going through walking motions; entirely unable to support either end of body; no response in any legs to pinching or pin prick; good eye reflexes. Photographed (Figs. 4, 5).

3:45 p. m. Died in spasms.

Autopsy: spleen small, light almost bloodless; liver dark, congested; kidneys dark, congested; gall bladder engorged with clear liquid; lungs normal.

G1.2 5/1. 7:00 a. m. Pig in upright position, but sprawled out, nose on floor, forelegs under body, hind legs extended backward; paralyzed. No reflex in legs under any stimulus.

11:00 a. m. Removed bandage and recovered 5 live ticks attached to posterior dorsal rump. Spasms increasing constantly; respiration shallow, rapid; walking motion of legs periodically; head drawn back over shoulder to the right almost to limit. Most exaggerated spasms seen to date; complete leg paralysis. Urine discharged at 11:18; clear.



- 11:38 a. m. Died in spasms.  
Autopsy: liver dark red, engorged; not mottled; gallbladder full of clear light amber liquid; spleen small, pale, bloodless; lungs congested, opaque; kidneys normal.
- Set G2. 4 guinea pigs infested with 20 nymphal female ticks each.  
5/5. No signs of effect to date. Series continued.
- Set G3. 4 guinea pigs infested with 5 adult, engorged female ticks each.  
G3.1 4/27. Found dead but still warm at 3 p. m. though apparently normal at noon.  
Recovered 5 engorged ticks. Much evidence of blood in tick feces. Ticks all attached on mid dorsal line in clipped area, but no sores present.  
Autopsy: liver normal in appearance; spleen small, almost bloodless; kidneys normal.
- G3.2 4/27. Quite normal this morning and feeding normally.  
3:00 p. m. Notably inactive, quiet.  
5:30 p. m. Breathing rapidly (102); complete paralysis in hind legs.  
11:30 p. m. Very weak; respiration 112. No blood in ear for smear. Respiratory symptoms similar to those of A2.  
4/28. 7:30 a. m. Still alive. Respiration 81, labored. Fore legs paralyzed.  
5:00 p. m. No change in condition up to this time.  
7:45 p. m. Died suddenly with only weak spasms.  
Autopsy: liver dark red, heavily engorged, and with a few pus pockets; gallbladder distended with clear colorless fluid; spleen small, almost bloodless; stomach with hemorrhagic spots; lungs normal; kidneys normal.
- G3.3 4/28. 3:00 p. m. Acting sluggish, though upright and in control of legs. Sits huddled in a ball and does not move. Apparently quite normal up to this time.
- 4/29. 7:30 a. m. Has not moved all night.  
10:15 a. m. Down on side, obviously dying. All legs going in walking movements periodically; can not maintain upright position; all coordination of legs gone; respiration almost indistinguishable.  
10:25 a. m. All reflexes except that of eye gone; no visible respiration; heart beating irregularly; completely paralyzed. Urine discharged clear.  
12:05 a. m. Died in spasms. Ticks clustered about spine at base of rump, all heavily engorged.  
Autopsy: Liver speckled, engorged; spleen small, light; almost bloodless; heart normal; gallbladder distended with light amber fluid; kidneys normal.
- Set G4. 1 guinea pig with mixed adult male and nymphal female ticks.  
5/6. No effect to date. Experiment continued.
- Set G5. 4 guinea pigs used as controls.  
5/6. All in fine shape. Continued.
- Set G6. 3 guinea pigs injected with emulsion made from mashed adult female ticks heavily engorged with moose blood. Solution of strained material in Ringer solution injected by hypodermic directly into heart on April 24.  
5/6. No effects noted to date. Series continued.
- SERIES H. One guinea pig injected with an emulsion of salivary glands of adult female ticks in Ringer solution. Injection by hypodermic subcutaneously over spine, on April 24.  
5/6. No effects noted to date. Series continued.



Summarizing the above experimental cases, certain definite symptoms are noted:

1. In almost every case paralysis of the legs is noted (Figs. 4, 5).
2. In many cases excessive activity is noted, the guinea pigs moving blindly, aimlessly.
3. Some pigs are affected rapidly, others more slowly. Those which die quickly show little activity, laziness, no fever, paralysis and death. Those which show delayed symptoms exhibit excessive activity, fever, diarrhea, paralysis and death.
4. Autopsies show an almost uniform engorgement of the liver, a reduction in size of the spleen and its almost bloodlessness.

Last September Dr. Cahn spent several days discussing with Mr. Hanson, who has seen first-hand many cases of moose dying of the disease, the external and internal characteristics of the disease as exhibited in infected moose. These may be summarized as follows:

1. Weakened, anemic condition, often with associated paralysis of the legs (Figs. 1, 2, 3).
2. Excessive activity, as witness the blind running about, bumping into obstacles.
3. Not all moose die of the disease, even though infected. A former guide told him how he had fed and cared for a diseased moose on Knife Lake for over a month, the animal eventually recovering; he showed photographs of the moose which practically duplicate those reproduced here. Hence in some cases apparently the disease is violent and fatal; in others slow and either fatal or not. Nothing is known as to possible relapse in moose. Fever is apparently present in the fatal cases, though no temperature readings are available.
4. Autopsies show internally an engorged liver and usually a reduced spleen.

By checking the two summaries above it will readily be seen that by transplanting the tick *Dermacentor albipictis* from infected moose upon normal guinea pigs and rabbits, the writers have induced a disease in these test animals expressed in exactly the same symptoms shown in the moose. Hence *Dermacentor albipictis* is shown as the transmitting agent of the disease. Furthermore it should be noted that the experiments (the G series) have to date shown no results of transmission of the disease through nymphal ticks, yet the percentage of transmission by both adult males and adult females has been high (75%).

#### DISCUSSION

Three obvious effects were produced by the introduction of *D. albipictis* from infected moose on to guinea pigs: a paralyzing effect

without fever, which appeared as early as the third day after the ticks had fed on the experimental animals, resulting in death; a high fever which appeared in ten days, with a gradual breaking down of the animal until terminated by death; a paralysis followed by apparently a complete recovery, this followed by a slow, steady relapse terminating in death. Blood smears from the peripheral blood stream (ear), and from the heart, spleen, liver and kidney were negative (G1.1, G3.1, G3.2, G3.3), but in the cases of A1 and A2 a few blood cells in blood smears from the ear showed blue bodies (Figs. 33-34), and bacteria.

About 50% of the erythrocytes from the spleen smears in cases A2 and G1.2 had these blue bodies, while they were notably fewer in liver smears. In about one half of the corpuscles of the liver smear in case A2 there appear from one to many intracellular bodies (Figs. 6-25). The present writers were struck by the similarity of these bodies to the piroplasms described in the voluminous literature on the subject. No attempt has been made to arrange the figures in a suggested developmental order as yet, but typical and outstanding examples have been selected for illustration. Figures 26, 27, 31 and 32 were taken from liver smears in case G1.2. Moore and Quick (1923) give the body temperature of the normal guinea pig as approximately 37.8° C. (about 100° F.). Case A2 had an apparently complete recovery from a severe attack of the disease ten days after being infected with the ticks. This was followed by another slight relapse and recovery, after which the pig relapsed a second time and died with a high fever (102.2° F.) thirty-eight days after the original infection with the organisms invading the erythrocytes. Case G1.2 died eight days after the introduction of *D. albipictis*, with only occasional organisms in the liver smears, but with numerous red granular inclusions (Figs. 28-29) in the liver and spleen smears. These bodies present a dark red granule with a halo in various positions in the erythrocytes and show a marked similarity to the description and figures of anaplasmosis of cattle given by Wenyon (1926). This authority also notes that they are frequently in association with piroplasmata. The difference in time of death in the two cases following infection may well account for the greater invasion of corpuscles in case A2. In cases A1, A2 and G1.2, blue bodies were numerous in the corpuscles in the peripheral blood stream before death, and very numerous in spleen and liver smears. This condition is found duplicated in cases of East Coast fever. Mason (1922) working on Egyptian fever of cattle, found Koch's blue bodies in the liver and lymphatic glands. He also reported ulcers in the abomasum, which we likewise have noted present in the stomach of case G3.2.

*Theileria mutans*, a piroplasmata of cattle, proves fatal in only 5 to 10% of cases infected. From all reports, the organism transmitted by

*D. albipictus* is fatal to probably considerably less than 3% of the moose population, most of which are probably tick infested to a greater or less degree, though there is no possible way of knowing at present what per cent of the animals infected with the disease succumb to it. That they may recover is shown by the specific case cited to Dr. Cahn. In our experimental animals, no hemoglobinuria is present as found in infections of *Babesia*. In *Theileria annulata* the parasite is persistent in the blood for long periods, and fatal relapses may occur at any time. Hadwen (1913) has reported a form of paralysis affecting sheep near Keremeos, British Columbia, and refers to numerous reports from British Columbia and Montana where other animals, such as colts, dogs, rabbits, grouse and even young children, developed paralysis within the six or seven day periods during which the ticks engorged upon them. The development of the disease is gradual. "The first noticeable sign is restlessness, the lambs at times stagger about and bump against obstacles and occasionally fall when trying to stop; later on they fall down and cannot rise; at this stage they struggle a great deal. As paralysis advances, the lambs cease struggling, but still have a wild-eyed look; they drink milk with less avidity. As soon as recovery begins, they again continually struggle in their attempts to rise, and this continues until they are able to stand, after which recovery is very rapid." It is interesting to compare these symptoms with those exhibited in our case A2. Hadwen further notes that the paralysis is usually of short duration, but that it may occasionally persist for long periods, and may terminate fatally. *Dermacentor venustus* was demonstrated by him to produce the above effects. He was not certain whether males in the absence of females could produce the paralysis, and suggested that other ticks may be capable of producing the same symptoms. Reported cases of paralysis show that if the ticks are removed the affected individuals quickly recover. Hadwen also points out that the ticks tend to congregate on the nape of the neck or along the back bone and dorsal rump, and suggests that this position may have some relation to the paralysis resulting from a bite near the central nervous system. In all our experiments we have found *D. albipictis* congregating along the dorsal ridge of the body in the immediate vicinity of the back bone. It may be pointed out that *D. albipictis* at least is strongly and negatively geotropic and will therefore tend to congregate and climb to the highest points on the animal unless they find a ready point for attack elsewhere.

The entire picture of this phase of the moose disease demonstrated by experiments of the writers in series A, B, C and G with *D. albipictis* as the infecting tick, bears out the suggestion of Hadwen that *D. venustus* may not be the only tick capable of producing "tick paralysis." This same phase of the disease is apparent in the descriptions of sick



moose and is clearly depicted for both moose and guinea pig in our figures. In the series G experiments, the ticks were assorted according to sex and maturity. The results to date demonstrate that both adult males and adult females produce the paralysis, and that the nymphal females do not produce paralysis. An emulsion of mashed, engorged, adult female ticks, injected into guinea pigs (series G6) and into rabbits (series E) have had no effect to date. One guinea pig, injected subcutaneously along the back with an emulsion of salivary glands of adult female ticks in Ringer's solution, has remained normal to date. The blood picture in cases of early death from paralysis was normal. All these findings are in accord with those reported for tick paralysis experiments by Hadwen (1913).

In all cases where the ticks were removed before twenty-four hours, as in series B and C, no harmful effects have been observed. These results correspond with the evidence produced by Nuttall and Hindle (1913) for a similar series of experiments with *Theileria parva*. They also found that emulsions of infected ticks and of salivary glands of ticks had no effect when inoculated into calves. No effects have been observed to date from similar injections into guinea pigs, as in series H.

The writers have isolated an organism from engorged female ticks which, when injected intravenously into guinea pigs and rabbits, produces all of the symptoms characteristic of the disease shown in moose: paralysis, profound cellular changes, death. The description of the organism and of additional experiments showing its behavior and virulence is to be published elsewhere.

#### EXPLANATION OF PLATE XVII

Fig. 1.—Diseased moose broken down in fore quarters.

Fig. 2.—Same moose showing paralysis in hind quarters.

Fig. 3.—Cow moose in weakened condition and with a heavy tick infection.

Fig. 4.—Guinea pig G1.2, showing complete paralysis of legs shortly before death. Also showing method of protecting ticks.

Fig. 5.—Same seen from the rear to show the sprawled out position of the paralyzed limbs.

THOMAS AND CAHN—DISEASE IN MOOSE



PLATE XVII

EXPLANATION OF PLATE XVIII

All drawings made with the aid of the camera lucida. Projected scale equals  $1.6\ \mu$ .

Figs. 6-25.—Erythrocytes (liver smears) of the guinea pig infected by the tick *Dermacentor albipictis* taken from a diseased dead moose. Case A2.

Figs. 26-27.—Erythrocytes from liver smears. Case G1.2.

Figs. 28-30.—Red bodies in erythrocytes from liver and spleen smears. Case G1.2.

Figs. 31-32.—Erythrocytes from liver smears. Case G1.2.

Figs. 33-34.—Blue bodies in corpuscles of spleen and liver smears. Cases G 1.2 and A 2.



THOMAS AND CAHN—DISEASE IN MOOSE



PLATE XVIII



## SUMMARY

1. An undescribed disease of moose in northern Minnesota is described.
2. Guinea pigs and rabbits, infested with the tick *Dermacentor albipictis* from diseased moose, have reproduced in detail the symptoms of weakness, anemia, paralysis, excessive blind activity and death as exhibited by infected moose, thus demonstrating the transmission of the moose disease through the tick.
3. The microscopic blood picture of the diseased moose and that of infected guinea pigs and rabbits are similar.
4. Tick paralysis is manifested in guinea pigs and rabbits as a result of the bite of *Dermacentor albipictis*, while animals which remove the ticks within twenty-four hours do not contract the disease.
5. Seventy-five per cent of the experimental animals upon which adult males and adult females of *Dermacentor albipictis* have gorged for over two days have died.
6. Blood smears of guinea pigs which present only the symptoms of paralysis are negative, while those animals having fever with the paralysis and which die in from seven to thirty-eight days, show blue bodies and red bodies in the erythrocytes from the spleen and liver as well as characteristic clumps of red staining bodies free in the blood plasma.
7. An organism causing all the symptoms of the disease has been isolated from ticks that had engorged on moose dying of the disease.

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## A NEW GENUS AND SPECIES OF MONORCHIDAE

LOWELL C. LLOYD AND JOHN E. GUBERLET\*

The parasitic fauna of marine fishes from Puget Sound waters is very slightly known and, as a consequence, presents an attractive field for research to anyone interested in parasitology. Since it is further true that knowledge in this phase of zoology for North America as a whole is inadequate and in an unsatisfactory condition, any contribution will be likely to have more than local interest. Linton, among the early writers, has undoubtedly done the most notable work in the field of marine fish trematodes. These works are of value chiefly as a survey of the field and are, therefore, necessarily superficial in many respects. While extensive, in that a large number of forms are described or reported, the descriptions of the species are in many cases incomplete, where not hopelessly inadequate, and the recognition of known forms uncertain. Among more recent works, that of Manter (1926) deals in a most thorough manner with a considerable number of species from a variety of hosts. This paper records the discovery of the occurrence of a new species of trematode belonging to the family Monorchidae Odhner 1911, which family has apparently been previously reported only once from American sources.

The parasite was found in the intestine of the common viviparous perch or "shiner," *Cymatogaster aggregatus* Gibbons. The fishes were secured from various localities: Peavine Pass, Davis Bay, False Bay, Squaw Bay, and Deer Harbor, in the San Juan Islands in the vicinity of the Oceanographic Laboratories during the summers of 1930 and 1931. Material was secured by washing out the intestinal contents of the fish, consisting chiefly of amphipods and copepods, and examining it with a binocular dissecting microscope. Nearly 100% infestation was found, only an occasional fish being free of the parasites. The number of trematodes in a single fish varied from one or two to a maximum of twenty one, the average being six or seven.

Due to the presence of large numbers of eggs and to the thick, spinous cuticula of the worm, little could be ascertained as to its anatomy in a living condition. Specimens were killed and fixed in hot Bouin's solution, stained and mounted in balsam. The most satisfactory whole mounts were stained in Mayer's paracarmine, while sections were stained as usual in hemotoxylin and eosin.

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\* Contribution from Zoological and Oceanographic Laboratories (No. 4) of the University of Washington. This is the first of a series of publications on the parasites of Puget Sound fishes by John E. Guberlet and his students.

The family is a small one. The only reference to its occurrence from American sources seems to be that of Linton (1910: 77-78) in his study on the helminth fauna of the Dry Tortugas. Nicoll (1915: 345) listed *Monorchis monorchis* (Stossich), *Proctotrema bacilliovatum* Odhner, *Lasiotocus mulli* (Stossich), and *Pristisomum pumex* Looss from British fishes. Later, Nicoll (1915a: 29-32) described two species belonging to the family which he assigned to the genus *Genolopa* Linton. Besides the paper of Odhner (1911: 247-253), in which he established the family, the most valuable references which the authors have consulted are Looss (1902: 115-118) with a description of *Monorchis monorchis* and *Monorchis parvus* and the very thorough morphological study on *Distomum perlatum* von Nordmann by the same author (Looss 1894: 24-33). This species is later redescribed by Looss (1899: 598) and the generic name *Asymphyrodora* assigned to it. Odhner's original description (1905) of the genus *Monorcheides* was not consulted, nor could any indication of the publication by Looss of a paper describing the genera *Lasiotocus* and *Pristisomum*, stated by Odhner (1911: 250) to be in manuscript at that time, be found. Brief descriptions of the genera are, however, given by Odhner who also defined the family and the two sub-families. He listed in them the genera as follows:

Sub-family Monorchinae

*Monorcheides* Odhner 1905

*Monorchis* (Monticelli) Looss 1902

Sub-family Proctotreminae

*Proctotrema* n.g.

*Lasiotocus* Looss in mnsr.

*Pristisomum* Looss in mnsr.

*Asymphyrodora* Looss 1899

Odhner (1911: 253) further stated that the species *Distoma tubulatum* Rudolphi belongs in the family Monorchidae but did not assign it to any genus. This was done by Poche (1925: 143) under the new generic name of *Physochoerus* in the sub-family *Monorchinae*. The genus *Genolopa* Linton is also classed with the Monorchidae but the two species described by Linton (1910: 77-78) are stated to belong to different sub-families. This genus will be considered in more detail later.

The present form, while definitely a Monorchid, does not comply strictly with Odhner's definition of either of the sub-families. It differs from the Monorchinae in the type of excretory system, in the presence of a seminal receptacle, in the posterior position of the yolk glands and in the median position of the testis. With respect to the Proctotreminae the only essential point of difference is in the extension of the intestinal

ceca to the posterior end of the body. However, it further differs from all described genera of Proctotreminae in the more posterior position of the yolk glands which lie in the posterior third of the body. These differences, while not of sufficient significance to exclude it from that sub-family, make it impossible to place it in any of the described genera and it is therefore proposed to establish a new genus for it to which the generic name *Telolecithus* is given.

TELOLECITHUS NOV. GEN.

Proctotreminae in which intestinal ceca extend to posterior end of body. Yolk glands behind ovary and genital complex. Oral sucker smaller than ventral sucker which lies about one third of body length from anterior end. Genital pore median, immediately anterior to ventral sucker. Testis median, just behind middle of body. Cirrus pouch large, reaching to or past anterior border of testis. Ovary smaller than testis and situated at its right antero-lateral border. Uterus opens laterally into metraterm near boundary of spined and unspined portions. Eggs small and shortly oval. Parasitic in intestine of marine fishes. Type and only species, *Telolecithus pugetensis* new species.

*TELOLECITHUS PUGETENSIS* n.g. and n.sp. [Figs. 1, 2]

The body form varies considerably with the state of contraction of the worm as, naturally, do the size relationships. The usual form in a moderately expanded specimen presents a tapering, neck-like portion, broadening in the region of the ventral sucker, until the greatest body width is reached shortly behind the ventral sucker, and then narrowing slightly to the bluntly pointed posterior end. Measurements, in balsam, of typical specimens give the following results: length 0.65 to 0.82 mm. and greatest width 0.28 to 0.37 mm. In completely extended living specimens the length may reach, or slightly exceed, 1 mm. while the greatest width varies from 0.22 to 0.30 mm. Besides varying with the state of contraction of the worm, the width is also somewhat dependent on the number of eggs present. The thick cuticula is closely set with small, pointed, curved spines which are directed posteriorly. They are most numerous in the anterior region, appear absent at the posterior end of the body, and are arranged in regular transverse rows, the spines in one row lying, not in line with, but between those of the preceding and following rows.

Numerous gland cells, "Hautdrüsen" of German authors, are present in the anterior region. They occur throughout the parenchyma and are numerous just within the muscular layers of the body wall. In the latter location they extend posteriorly nearly to the posterior end of the body. In no case could they be associated with any of the organ systems of the animal.



The oral sucker is terminal but its opening is directed somewhat ventrad. Its transverse diameter varies from 0.08 to 0.11 mm. In living specimens the longitudinal diameter is usually a little greater while in balsam the outline may be slightly transversely ovate. Situated about one third of the body length from the anterior end is the ventral sucker which is transversely ovate in balsam but nearly circular in living specimens, and measures 0.12 to 0.15 mm. by 0.10 to 0.14 mm. It is to a large extent depressed within the body of the worm so that in living specimens it frequently appears quite small.

The oral sucker is pierced at its base by the very short, thin walled prepharynx (Fig. 3) followed by the small, oval pharynx which measures 0.03 to 0.04 mm. by 0.05 to 0.65 mm. The esophagus is very short, 0.04 to 0.05 mm. long, and following it the intestinal ceca diverge laterally, pass around the ventral sucker, and then continue a nearly straight course along the lateral margins to the posterior end. Posteriorly their diameter increases considerably.

The excretory pore is terminal and from it the sac-shaped excretory vesicle passes a short distance anteriorly between the tips of the intestinal ceca. From the anterior tip of the vesicle two stems diverge laterally and pass anteriorly a little ventral and medial to the intestinal ceca until in the region of the ventral sucker they leave the ceca and continue a sinuous course to about the level of the pharynx. The size of the vesicle varies considerably, the length from 0.10 to 0.16 mm. and the width from 0.06 to 0.08 mm.

The single, large testis is median, its anterior border being somewhat behind the middle of the body and it extends posteriorly a short distance into the caudal third. Its shape is quite variable but always slightly lobed and broader than long. Frequently the shape is roughly triangular with the broadest part directed anteriorly. It measures 0.13 to 0.15 mm. for its greatest breadth and 0.09 to 0.13 mm. in length. No afferent duct or ducts could be detected in any of numerous series of sections.

A large and prominent cirrus pouch lies medial and dorsal to the ventral sucker and extends some distance behind its posterior border and turns to the left, reaching to or past the anterior border of the testis. Its length is from 0.25 to 0.35 mm. and greatest width 0.06 to 0.07 mm. In its base is an oval seminal vesicle which measures 0.07 by 0.04 mm., its long axis being parallel with the longitudinal axis of the cirrus pouch. Lining the seminal vesicle is a single layer of columnar cells with prominent nuclei and the vesicle is usually filled with sperm cells. Following the seminal vesicle and joining it with the cirrus is a short pars prostatica with very muscular walls giving it a globular form with a diameter of about 0.05 mm. The cirrus forms a rather long, wide tube with muscular walls and throughout its length thickly

set with narrow pointed spines. On the medial or left side of the cirrus the spines measure 14 to 18 $\mu$  in length and are about 3 $\mu$  in diameter at the base while the spines on the opposite wall are a little shorter and broader at their bases. Numerous large prostate cells radiate from the pars prostatica and fill most of the remaining space in the cirrus pouch. At about the level of the anterior margin of the ventral sucker the metraterm opens into the left side of the cirrus following which a common, unspined genital atrium turns sharply ventrad and continues to the genital pore.

The ovary is considerably smaller than the testis and like it of somewhat variable shape but usually three lobed and measures 0.08 to 0.12 mm. along its greatest diameter which is transverse. It is situated anteriorly and to the right of the testis, its posterior border being closely approximated to the anterior border of the testis. On the antero-dorsal and somewhat lateral margin of the ovary is a small outpocketing, giving rise to the oviduct which passes anteriorly for a short distance and then turns sharply medially and posteriorly. Shortly after turning, the oviduct gives off Laurer's canal which passes dorsally and medially behind the cirrus pouch but could not be traced to an actual opening on the dorsal surface. From the base of Laurer's canal is given off a very small seminal receptacle which lies along the lateral border of the cirrus pouch. It appears to be invariably devoid of contents, is roughly spherical in outline, and measures from 0.015 to 0.02 mm. in diameter.

Beyond the origin of Laurer's canal the oviduct continues farther posteriorly to nearly the anterior level of the testis where it turns to the left and passes between the testis and cirrus pouch to the left side of the testis where it enlarges to form the ootype (Fig. 4) and then turns posteriorly and medially around the testis. The oviduct and associated structures are so compressed between the cirrus pouch and testis and their relationships further distorted by the presence of large numbers of eggs in the uterus that the structure of the complex can only be made out with difficulty.

The yolk glands, composed of six or seven small follicles on each side, lie along the lateral margins in the posterior third of the body. Individual follicles measure 0.04 to 0.05 mm. in diameter. Short ducts from each follicle unite to form a thick yolk duct which passes anteriorly just dorsal to the intestinal ceca to the anterior margin of the testis where it turns sharply mediad and joins the duct from the opposite side to form a yolk reservoir near the midline. The yolk reservoir lies just dorsal to and a little below the oviduct into which it opens. Partially surrounding the ootype and the oviduct for some distance are the cells of the shell or Mehlis' gland. Toward the right they are in close contact with the ovary and in some sections are difficult to separate from it

since they stain rather similarly and when cut at the proper angle have about the same shape as the ova.

The oviduct, after passing around the testis, continues posteriorly as the uterus which fills the post-testicular space between the intestinal ceca and also all available space lateral to testis and ovary, reaching on either side to about the middle of the ventral sucker and overlapping, to some extent, the borders of the testis and ovary. Masses of sperm cells frequently occur in the ootype and more proximal portions of the uterus. The uterus finally opens laterally into the metraterm on its medial side at the extreme terminal part of the spined portion. In this connection it may be mentioned that Looss (1902: 117) gives the opening of the uterus into the metraterm as at the boundary between the spined and unspined portions which statement is contested by Odhner (1911: 248) who says that the opening occurs in the terminal part of the spined portion. In the present form the actual opening is within the spined portion although the point at which the uterus begins to penetrate the wall of the metraterm lies somewhat in the posterior, unspined region.

The metraterm is a muscular sac about two thirds the length of the cirrus pouch into which it opens near the anterior margin of the ventral sucker. It is divided into a posterior, sac-like, unspined portion which comprises about one third of its length and a narrower, anterior portion with muscular walls set with spines which are narrower but of about the same length as the longer cirrus spines and are directed posteriorly throughout most of the length of this portion. The length of the metraterm is from 0.19 to 0.21 mm. and its greatest width 0.04 to 0.045 mm. anteriorly, and 0.06 to 0.065 mm. in the posterior portion. The eggs (Fig. 5) are yellow to light brown in color and shortly oval. They measure 18 to 20 $\mu$  by 11 to 12 $\mu$ .

This species is parasitic in the intestine of the viviparous perch or "shiner" *Cymatogaster aggregatus* Gibbons.

#### DISCUSSION OF THE GENUS GENOLOPA LINTON

It has previously been mentioned that the genus *Genolopa* Linton was included by Odhner in the family Monorchidae. This genus was established by Linton (1910: 77-78) and included with four other genera in the family Siphoderidae chiefly on the basis of the possession of a genital sucker as Linton interpreted the ventral sucker of *Genolopa*. Odhner (1911: 252) calls attention to the heterogeneous character of the family Siphoderidae and also to the fact that the genus *Genolopa* belongs in the family Monorchidae. He further states that of the two species described by Linton, one, *Genolopa ampullacea*, the type species, belongs in the sub-family Monorchinae and the other, *Genolopa truncata*,

in the sub-family Proctotreminae. Poche (1925:143-144) includes *Genolopa* as one of the genera of Monorchidae.

*Genolopa ampullacea*, except for the fact that the yolk glands are slightly posterior to the ventral sucker and a seminal receptacle is stated to be present, appears to belong typically to the Monorchinae. It is possible that Linton was mistaken in describing a seminal receptacle as present since the ootype and more proximal portions of the uterus may contain masses of sperm cells and could be misinterpreted as a seminal receptacle. Should it be shown that a seminal receptacle is actually absent in *Genolopa ampullacea* any doubt as to the proper position of the species in the sub-family Monorchinae would be removed. *Genolopa truncata*, providing Linton's figure showing the intestinal ceca ending considerably before the posterior end is correct, conforms in all respects to Odhner's diagnosis of the Proctotreminae.

Nicoll (1915a: 29-32) described two species of trematodes which he assigned to the genus *Genolopa* Linton, viz., *Genolopa trifolifer* and *Genolopa cacuminata*. In both, the intestinal ceca are stated to extend to the posterior end of the body although Nicoll's figures, as Linton's for *Genolopa truncata*, show them only about as far as the middle of the body. A seminal receptacle is stated to be absent in both species. Both species are large for Monorchidae, *Genolopa trifolifer* having a length of 1.5 to 2.1 mm. and *Genolopa cacuminata* 2.1 to 3.25 mm. *Genolopa cacuminata* is further peculiar in the narrow, elongated shape of the body, in the possession of a long prepharynx and in the less compact and atypical arrangement of the yolk glands. Neither Nicoll nor Linton mention the excretory system of any of the species of *Genolopa*.

Both of Nicoll's species show characteristics of the Proctotreminae, in the median position of the testis, position of the yolk glands posterior to the ventral sucker and the largely post-testicular position of the coils of the uterus. The body shape is also more characteristic of the Proctotreminae than it is of the Monorchinae. However, the absence of a seminal receptacle and the posterior extension of the intestinal ceca are characters of the Monorchinae. Considering the small size of the seminal receptacle in the Monorchidae, it is possible that it was overlooked by Nicoll. In this case the essential difference between the two species and the Proctotreminae as defined by Odhner, except for those characters in which *Genolopa cacuminata* is somewhat atypical of the family as a whole, would be reduced to the extension of the intestinal ceca to the posterior end of the body in which respect they would stand with *Telolecithus pugetensis*.

Without more detailed information concerning the morphology of the several species of *Genolopa*, especially as to the type of excretory system and the presence or absence of a seminal receptacle, any revision





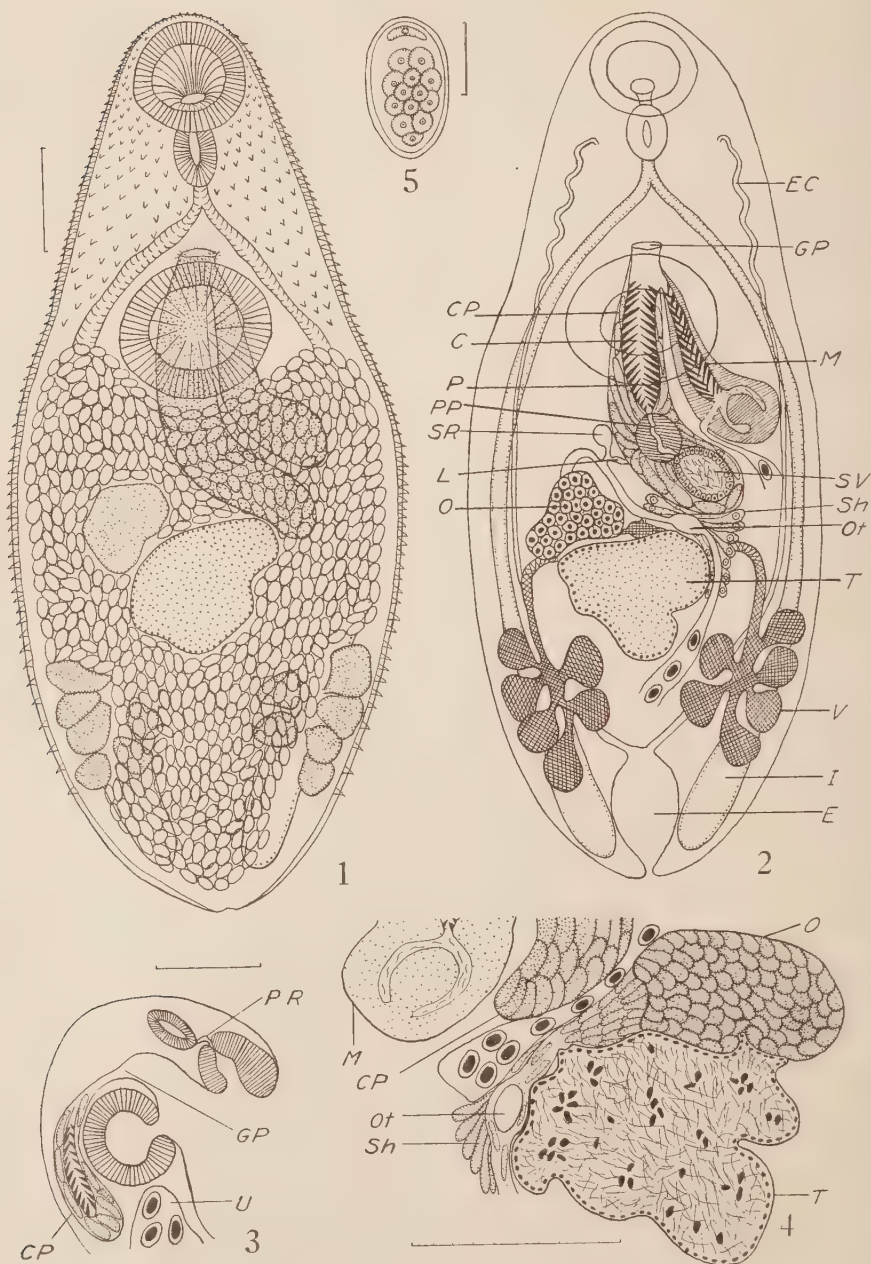


PLATE XIX

of the genus is, of course, impossible. That *Genolopa ampullacea* belongs in the sub-family Monorchinae and *Genolopa truncata* in the Proctotreminae appears certain. Nicoll's two species are probably Proctotreminae but differing generically from *Genolopa truncata* Linton and possibly represent two distinct genera. Both appear to have their closest affinity with *Telolecithus pugetensis* and with it occupy a somewhat intermediate position between the Monorchinae and Proctotreminae.

## SUMMARY

1. *Telolecithus pugetensis* is described as a new species and the type of a new genus, belonging to the family Monorchidae Odhner.
2. The parasite came from the intestine of the viviparous perch, *Cymatogaster aggregatus* Gibbons.
3. The genus *Genolopa* Linton is discussed.

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## EXPLANATION OF PLATE XIX

All drawings made with aid of camera lucida. The scale in all figures equals  $100\mu$  except figure 5 in which it is  $10\mu$ .

- Fig. 1.—Ventral view of *Telolecithus pugetensis*.  
 Fig. 2.—Semi-diagrammatic reconstruction.  
 Fig. 3.—Sagittal section through anterior region.  
 Fig. 4.—Frontal section through region of shell gland complex; dorsal view.  
 Fig. 5.—Egg from distal portion of uterus.

## ABBREVIATIONS USED

C, cirrus; CP, cirrus pouch; E, excretory vesicle; EC, excretory canal; GP, genital pore; I, intestinal cecum; L, Laurer's canal; M, metraterm; O, ovary; Ot, ootype; P, prostate cells; PP, pars prostatica; PR, prepharynx; Sh, Mehlis' or shell gland; SR, seminal receptacle; SV, seminal vesicle; T, testis; V, yolk gland.

## A COMPARATIVE STUDY OF THE EGGS OF CALIFORNIAN ANOPHELINES

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The eggs of *Anopheles pseudopunctipennis* Theobald have been described by Herms and Freeborn (1920) as possessing an encircling collar produced by a flaring out of the membranous cover of the egg. Later observations made on eggs deposited by this species in our laboratories in Berkeley indicated a rather marked divergence from the above description, for which reason a study of the eggs of this species was renewed and in addition a detailed comparative study of the eggs of the other species of Californian anophelines was undertaken as well, namely, *Anopheles maculipennis* Meigen and *A. punctipennis* Say.

Gravid female anophelines were collected in the field, brought to the laboratory and placed individually in wine glasses about one-fourth full of distilled water. The glasses were covered with bobbinet to prevent escape but provided with a hole plugged with cotton. These were kept in a room where the temperature ranged from 66° to 75° F., with relative humidity at from 35 to 60 per cent.

The majority of the mosquitoes oviposited during the early part of the second night, others not until the fourth or fifth. The eggs were removed from the containers with a camel's-hair brush and transferred to slides where they were studied microscopically in a drop of distilled water or in Gater's solution. Transmitted and reflected artificial light was used. Gater's solution was found to be particularly useful. The formula is as follows:

Water, distilled .....	10 per cent
Gum arabic, picked .....	8 per cent
Chloral hydrate .....	74 per cent
Glucose syrup .....	5 per cent
Acetic acid, glacial .....	3 per cent

Heretofore, Herms (1929) and Herms and Freeborn (1920) have given the number of eggs deposited by California anophelines as follows: *Anopheles maculipennis*, maximum for one female 385, average per female 203; *A. punctipennis*, maximum for one female 321, average per female 203; *A. pseudopunctipennis*, maximum for one female 157, average per female 106.

Our records for the present season (1931) show the following: *Anopheles maculipennis*, maximum for one female 288, average per female 195; *A. punctipennis*, maximum for one female 298, average per female 202; *A. pseudopunctipennis*, maximum for one female 210, average per female 151.



*Anopheles pseudopunctipennis* THEOBALD

The length of the eggs exclusive of the floats ranges from 493 to 510 $\mu$ ; inclusive of floats, from 510 to 544 $\mu$ . The upper or dorsal surface is nearly plane and the lower or ventral surface markedly convex. Both ends of the egg are rounded, the anterior end being the broader. Floats are not only present as shown by Herms and Freeborn (1920) but completely encircle the egg which lies flat upon the surface of the water with the floats in nearly every instance extended (Fig. 1). The floats in newly deposited eggs function so efficiently that it is almost impossible to turn them over and even with the older eggs much manipulation is required to turn them on their sides (Figs. 3 and 4).

A number of eggs were encountered in which the floats were folded inward upon the surface of the egg (Fig. 2) which might easily give the appearance of a collar-like float as described by Herms and Freeborn. In figure 4 where the posterior end of the egg protrudes above the "collar" of the float is shown an aspect that corresponds rather closely with the description given by the above authors. The floats average from 45 to 47 in number, and from 15 to 25 $\mu$  in width, being wider along the sides of the egg and narrower at the ends. In figure 4 their structure is shown in detail and it is seen that they are composed of sections with numerous small air cells, each section being separated from its neighbor by a clear membranous compartment which also appears to contain air.

When viewed by transmitted artificial light the eggs are a sepia brown in color with sculpturings upon the shell in the form of minute depressions which are visible after it has been denuded of its ensheathing membrane (Fig. 8). With proper magnification it is seen that the delicate membrane covering the egg gives a finely granular appearance to the dorsal surface (Fig. 1) while the reticulum over the ventral surface has a definite polygonal pattern (Fig. 6) produced by beads of gelatinous material which fit into the depressions on the surface of the egg shell (Figs. 7 and 8).

Just below the apex of the egg on the ventral surface lies the micropyle (Fig. 7) which, like those of *A. maculipennis* and *A. punctipennis*, resembles a "daisy" with from seven to eight "petals" surrounding a center containing a hole or canal, surrounded in turn by light and dark rings. How the reticulum of the ventral surface "fits" over the micropyle may be seen in figure 6. When mounted with a cover glass both hatched and unhatched eggs are unevenly divided into thirds or sometimes fourths by dark transverse bands which are evidently due to creases caused by pressure (Fig. 8). The eggs of *A. pseudopunctipennis* lack both the "frill" along the margins and the globular structures found at the apices of the majority of anopheline eggs.

*Anopheles maculipennis* MEIGEN

The description of these eggs given by Herms and Freeborn (1920), as well as those by Nuttall and Shipley (1901) and Christophers and Stephens, upon whose work many other authors have based their descriptions, tallied with our findings in the following respects. The eggs are fusiform or boat-shaped with rounded ends, the anterior end being the broader. The upper surface is flattened with a slight concavity while the lower is convex. The ventral surface is granular while the dorsal surface is finely reticular (Fig. 11). Along both margins of the dorsal surface of the egg runs a fluted frill or ribbed rim which is discontinued at the floats. The floats (Figs. 9, 10 and 11) are medianly placed and consist of from 12 to 13 compartments. Neither the "frill" nor the floats resemble in structure the floats of *A. pseudopunctipennis* as they lack the minute air chambers of the latter and are composed of simple compartments without any pattern. When viewed by transmitted artificial light this egg is also a sepia brown and when under a cover slip shows the transverse grooves or wrinkles found in *A. pseudopunctipennis*. The sculpturing upon the shell of *maculipennis* is much more pronounced, however, and the pits or depressions are larger and more irregular (Fig. 12).

Howard (1900) and Blanchard refer to from five to seven, minute, dark, circular spots at each end of the egg, while Hehir quotes Newstead in regard to anopheline eggs in general as follows, "There is a micropylar process at the end of the egg encircled by a relatively large rosette formed by a material precisely similar to that which forms the lateral floats." Upon careful examination of the ends of the egg with ocular #20 and the oil immersion lens (1.9 mm.) we made the following discoveries, i. e., the dark spots referred to by a number of observers are definite structures varying from five to eight in number. They are globular in form and are "pronged" at the top like the setting of an old-fashioned ring (Figs. 12 and 13). Within the prongs there is a globe of gelatinous material. The same delicate membrane which covers the egg extends up to and perhaps is attached to these globular structures. The micropylar process to which very few observers have referred is similar to that of *pneusopunctipennis* (Fig. 14). This figure shows the comparative size of the end "bulbs" and the micropyle and to the left the micropylar covering in the membrane which has been shifted from its proper position.

*Anopheles punctipennis* SAY

The eggs of *A. punctipennis* ranged from 534 to 578 $\mu$  in length and differed markedly from those of *A. maculipennis* in the "frill" which here extends along the margins of the egg without any interruption at the site of the floats (Fig. 15). The latter are located on the upper

portion of the ventral surface of the egg (Fig. 17), extend farther along the sides of the egg than do those of *A. maculipennis* (Fig. 16), and consist of a greater number of compartments which range from 16 to 22 in number and which do not converge in the same fan-wise fashion as do those of *A. maculipennis*.

The "granulation" of the dorsal surface is somewhat more delicate than that of *A. maculipennis* and the membrane covering the ventral surface is entirely different from that of the latter, closely resembling that of *A. pseudopunctipennis* (Fig. 18). The sculpturing of the egg-shell itself (Fig. 19) closely resembles that of *A. maculipennis* in the size and irregularities of the depressions. As shown in figures 19 and 20, there are terminal globular structures from five to eight in number differing from those of *A. maculipennis* only in that the "prongs" seem more pointed and their rims are slightly but definitely serrated. The micropyle (Fig. 20) resembles that of the other two species. It is here shown as one sees it looking through the dorsal surface.

#### SUMMARY

The eggs of the three species of Californian anophelines differ so markedly from one another that characters of their external anatomy may be used to differentiate the species.

#### ACKNOWLEDGMENT

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## EXPLANATION OF PLATE XX

Ova of *Anopheles pseudopunctipennis* Theobald

Fig. 1.—Egg as it normally rests upon the surface of the water. (About 75  $\times$ .)

Fig. 2.—Egg with the floats turned inward. (About 75  $\times$ .)

Fig. 3.—Egg turned upon its side showing the polygonal reticulum upon the ventral surface. (About 75  $\times$ .)

Fig. 4.—Hatched egg lying upon its side with posterior end extending beyond the floats. (About 75  $\times$ .)

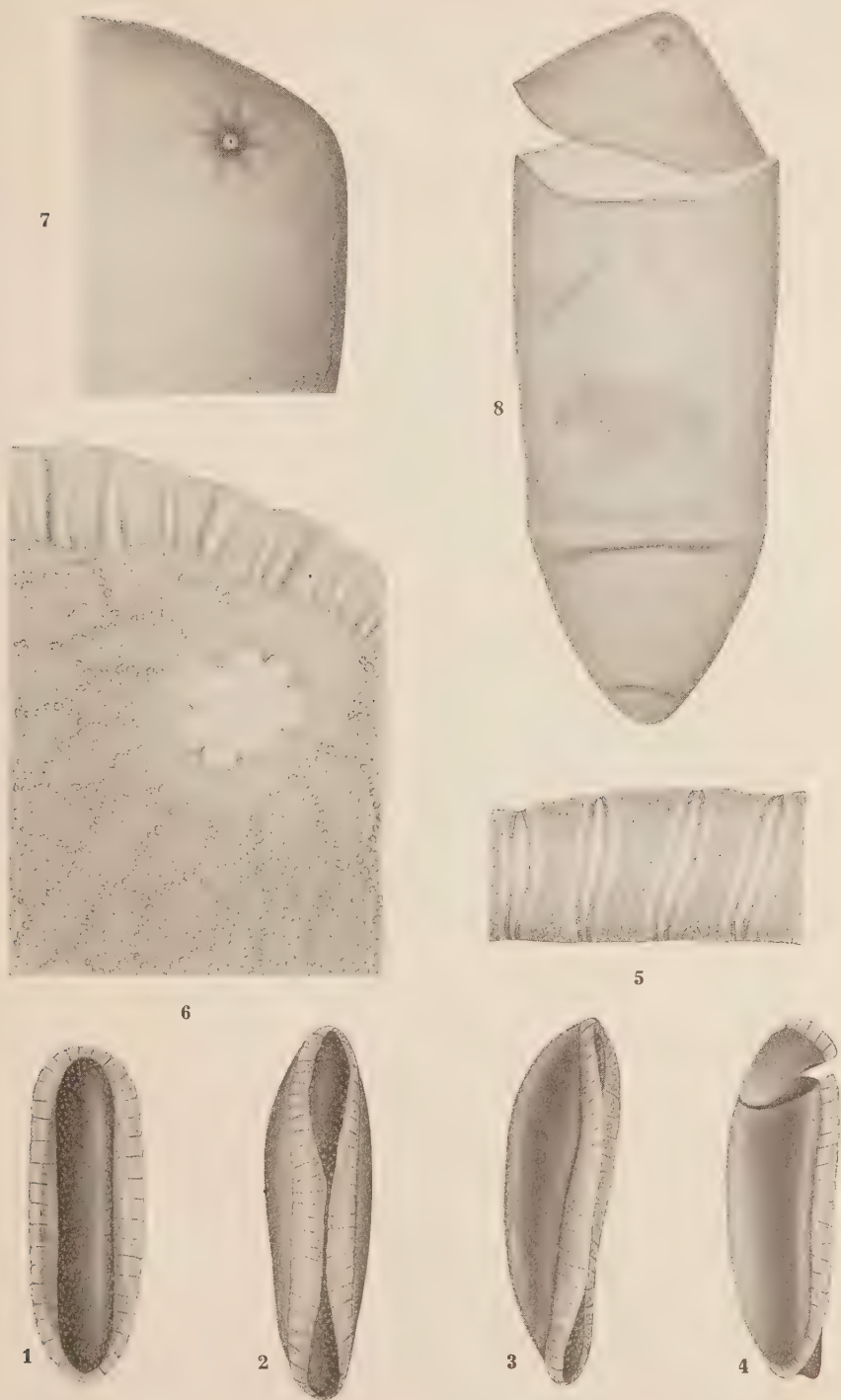
Fig. 5.—Floats with the minute air chambers. (About 440  $\times$ .)

Fig. 6.—A view of the membrane torn from the ventral surface of the egg showing the region about the micropyle. (About 900  $\times$ .)

Fig. 7.—View showing the micropyle and the sculpturing of the shell. (About 900  $\times$ .)

Fig. 8.—Hatched egg shell showing transverse creases, micropyle and sculpturing. (About 200  $\times$ .)





EXPLANATION OF PLATE XXI

Ova of *Anopheles maculipennis* Meigen.

Fig. 9.—Egg as it rests normally upon the surface of the water, showing the frill, the floats and the granulation of the dorsal surface. (About 75  $\times$ .)

Fig. 10.—Side view of the egg showing the frill interrupted by the floats. (About 75  $\times$ .)

Fig. 11.—Ventral surface of the egg. (About 75  $\times$ .)

Fig. 12.—The “end bulbs” and the sculpturing upon the egg shell. (About 1950  $\times$ .)

Fig. 13.—One of the “end bulbs.” (About 1,950  $\times$ .)

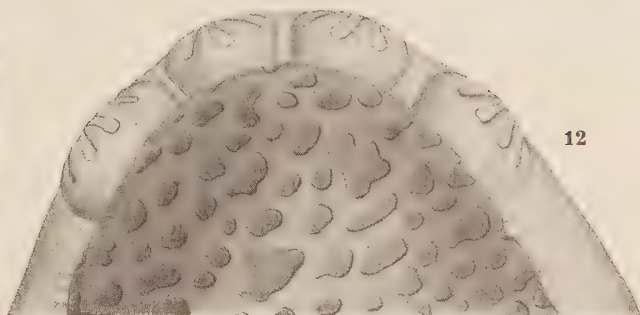
Fig. 14.—The micropyle and to the left the opening in the membrane which surrounds the micropyle. The membrane has been shifted from its normal position. (About 900  $\times$ .)



14



13



12



9



10



11

EXPLANATION OF PLATE XXII

Ova of *Anopheles punctipennis*, Say.

Fig. 15.—The egg as it rests normally upon the surface of the water, showing the delicate granulation of the dorsal surface. Note the frill is continuous. (About 75  $\times$ .)

Fig. 16.—The egg with the frill turned inward. (About 75  $\times$ .)

Fig. 17.—The egg turned upon its side showing the position of the floats. (About 75  $\times$ .)

Fig. 18.—Ventral view of egg showing the polygonal reticulum. (About 75  $\times$ .)

Fig. 19.—The "end bulbs" and the sculpturing of the egg shell. (About 1,950  $\times$ .)

Fig. 20.—The micropyle as seen through the dorsal surface. (About 900  $\times$ .)



*HERMS AND FROST—CALIFORNIAN ANOPHELINES*



20



19



15



16



17



18



# VIABILITY AND RATE OF DEVELOPMENT OF THE EGGS AND LARVAE OF THE TWO PHYSIO- LOGICAL STRAINS OF THE DOG HOOK- WORM, *ANCYLOSTOMA CANINUM*\*

A. O. FOSTER AND S. DAENGSVANG

The discovery (Scott, 1929, 1929a) of the existence of two physiological strains of the dog hookworm has been of interest in human helminthology because of the analogies that exist between them and certain human helminths. The two strains of *A. caninum*, specific to the dog and cat respectively, lend themselves to experimental procedures which are impossible with such physiological strains as the human and pig roundworm and whipworm. In addition to this, the great numbers of morphologically indistinguishable species and strains of parasites which are found in two or more hosts make a study of differences between two physiological strains of interest to parasitologists generally.

The work of Scott (1928, 1930) and McCoy (1931) has served to indicate many differences between the cat and dog strains of *A. caninum*, which for purposes of brevity are reviewed here in tabular form:

Dog Strain of <i>A. caninum</i>	Cat Strain of <i>A. caninum</i>
1. Average development in dogs 60 puppies— $44.7 \pm 1.8$ per cent 38 young dogs— $41.6 \pm 2.2$ per cent 9 old dogs— $2.6 \pm 1$ per cent	1. Average development in cats 19 kittens— $46.5 \pm 3$ per cent 6 mature cats— $13 \pm 2$ per cent
2. Average development in cats 35 kittens— $1.92 \pm 0.45$ per cent 19 mature cats— $0.49 \pm 0.28$ per cent	2. Average development in dogs 17 puppies— $0.51 \pm 0.2$ per cent
3. Average daily egg production of females in normal host (dog) $16,077 \pm 1,044$	3. Average daily egg production of females in normal host (cat) $2,350 \pm 104$
4. Average daily egg production of females in cat host $2,340 \pm 583$	4. Average daily egg production of females in dog host 11,600 (3 dogs)
5. Prepatent period about 14 days	5. Prepatent period about 17 days
6. Average length of 630 females 11.28 mm. Average length of 539 males 8.86 mm.	6. Average length of 253 females 8.42 mm. Average length of 240 males 6.96 mm.

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\*From the Department of Helminthology of the Johns Hopkins University School of Hygiene and Public Health. This work was made possible through the cooperation of the International Health Division of the Rockefeller Foundation. The writers are indebted for many helpful suggestions to Dr. W. W. Cort, under whose supervision the work was carried out.

Dog Strain of <i>A. caninum</i>	Cat Strain of <i>A. caninum</i>
7. Host-specificity not altered by passage through cat host †	7. Host-specificity reversed by passage through single dog host †
8. Infestations often large	8. Infestations usually small and never as heavy as heavy infestations in dogs
*9. Percentage hatching of eggs 89 ± 2 at 21°C. 50 ± 6 at 31°C.	*9. Percentage hatching of eggs 28.5 ± 2 at 21°C. 20 ± 2 at 31°C.
*10. Percentage development of eggs to infective larval stage 44 ± 3 at 21°C. 47 ± 2 at 31°C.	*10. Percentage development of eggs to infective larval stage 5 ± 0.8 at 21°C. 6 ± 0.5 at 31°C.
*11. Percentage development of hatched larvae growing to infective stage 49 ± 4 at 21°C. 94 ± 6 at 31°C.	*11. Percentage development of hatched larvae growing to infective stage 17 ± 2 at 21°C. 30 ± 2 at 31°C.

\* Points 9, 10, and 11 are advanced in this paper but have been included in the table for completeness. These results when analyzed statistically show differences of the means divided by the probable error of the differences ranging from 5 to 21, which is highly significant.

† Scott (1930) found that the dog strain retained its infectivity for dogs and did not become more infective to cats, even after its passage through three successive generations of cat hosts. In a single experiment, however, in which the cat strain was passed through a dog host, the strain obtained gave a 20 per cent development in one dog infected and only 5 per cent in one cat, thus indicating a reversal of specificity. This observation has been recently confirmed by Foster and Cort in this laboratory, with even more striking results. Larvae obtained from one of several dogs infected with the cat strain were given to two dogs and one cat. No development was obtained in the cat, while the dogs showed 30 and 80 per cent development respectively. This strain was subsequently carried through six successive generations of cat hosts, without apparently decreasing its specificity for dogs or increasing its specificity for cats.

The discovery of McCoy (1929, 1929a) that hookworm larvae feed entirely upon bacteria, and can be grown in pure cultures of bacteria has made it possible to study the developing eggs and larvae of the two physiological strains of *A. caninum* under controllable conditions. Eggs cultured by this method can be treated in every way in which a bacterial culture can be treated, thus eliminating the effects of fecal contamination, variations in temperature, and other influences. Consequently, under the same environmental conditions it has been possible to compare the relative proportions of the eggs of these two strains which hatch and to ascertain their relative potentialities for development to the infective stage.

One dog (D 674) and two cats (C 473 and C 488) were used as culture animals. The dog strain is the same as that designated DO by Scott (1929b) and carried in this laboratory since 1925. The cat strain is the same as that designated C 1 by Scott and carried in this laboratory since 1925. Eggs were isolated from the feces after the method of McCoy (1929a:142) by a series of water sedimentations, centrifugation,



gation, and salt flotation. For determining the rate and number hatching, a method commonly used in this laboratory was employed, which consists in placing a known number of eggs in a drop of water on a micro slide, encircling the area with a vaseline ring sufficient to support and seat a 22 mm cover slip. The slides were then incubated at room temperature, 21°C., 31°C., and 37°C. Each day the number of hatched larvae was counted. For determining the percentage development of eggs to infective larvae, the methods outlined by McCoy for culturing

TABLE 1.—Percentage Hatching of Eggs of the Dog Strain of *A. caninum*

Exp. No.	Number Eggs Cultured	Percentage Hatching at Various Temperatures for Time Intervals											
		17 to 26 Hours				42 to 48 Hours				67 to 72 Hours			
		Room Temp.	21° C.	31° C.	37° C.	Room Temp.	21° C.	31° C.	37° C.	Room Temp.	21° C.	31° C.	37° C.
1	59	17	..	..	..	48	..	..	..	80	..	..	..
	84	12	..	..	..	78	..	..	..	93	..	..	..
	54	12	..	..	..	46	..	..	..	85	..	..	..
	83	15	..	..	..	62	..	..	..	74	..	..	..
2	140	..	82	..	..	..	—	..	..	83	..	..	..
	20	..	90	..	..	..	..	..	..	100	..	..	..
	106	..	69	..	..	..	..	..	..	77	..	..	..
	76	..	90	..	..	..	—	..	..	92	..	..	..
3	513	..	..	21	..	..	..	72	..	..	..	24	..
	288	..	..	27	..	..	..	88	..	..	..	31	..
	695	..	..	25	..	..	..	76	..	..	..	29	..
	582	..	..	24	..	..	..	64	..	..	..	37	..
4	612	..	..	..	17	..	..	..	13	..	..	..	7
	569	..	..	..	24	..	..	..	18	..	..	..	11
	754	..	..	..	45	..	..	..	20	..	..	..	9
	612	..	..	..	24	..	..	..	17	..	..	..	10
5	140	43	..	..	..	55	..	..	..	71	..	..	..
	78	38	..	..	..	79	..	..	..	91	..	..	..
6	68	..	87	..	..	..	—	..	..	89	..	..	..
	64	..	79	..	..	..	—	..	..	86	..	..	..
7	146	..	..	82	..	..	..	93	..	..	..	71	..
	151	..	..	80	..	..	..	90	..	..	..	82	..
8	318	..	..	..	83	..	..	..	67	..	..	..	51
	132	..	..	..	82	..	..	..	46	..	..	..	43
9	73	27	..	..	..	54	..	..	..	91	..	..	..
10	42	..	80	..	..	..	83	..	..	..	96	..	..
11	44	..	..	50	..	..	..	91	..	..	..	78	..
12	66	..	..	..	61	..	..	..	51	..	..	..	9
Average....		24	82	45	48	60	83	82	33	84	89	50	20

hookworm larvae with bacteria were followed throughout. Cultures were made up in 250 cc Erlenmeyer flasks which were stoppered with cotton plugs, and consisted of 15 to 20 cc of agar diluted with three parts of water. The cultures were autoclaved and inoculated with bacteria 24 hours before the eggs were introduced. The flasks were incubated for 10 days at temperatures of 21 °C. and 31 °C., at which time the larvae were isolated by means of a miniature Baermann apparatus. Ordinary bacteriological agar and inoculations of *Bacillus coli* were supplied by the Department of Bacteriology of this institution.

A study of the viability of the eggs of the cat and dog strains of *A. caninum* presented striking differences. The percentage of the eggs which hatched was accepted as the criterion of viability. Table 1 and

Table 2, for the dog and cat strains respectively, present the data affecting viability. The dog strain eggs showed a greater viability at every temperature studied. The eggs incubated at room temperature were comparable to those at 21°C., although the former temperature was subject to greater fluctuation. At room temperature the eggs of both strains showed increased hatching for each day of the three days of hatching, the eggs of the dog strain reaching a maximum of 84 per cent as compared to 34 per cent for the cat strain. At 21°C. the same

TABLE 2.—*Percentage Hatching of Eggs of the Cat Strain of A. caninum*

Exp. No.	Number Eggs Cultured	Percentage Hatching at Various Temperatures for Time Intervals											
		17 to 26 Hours				42 to 48 Hours				67 to 72 Hours			
		Room Temp.	21° C.	31° C.	37° C.	Room Temp.	21° C.	31° C.	37° C.	Room Temp.	21° C.	31° C.	37° C.
1	148	5	..	..	..	38	..	..	..	46	..	..	..
	174	4	..	..	..	39	..	..	..	46	..	..	..
	28	3	..	..	..	34	..	..	..	37	..	..	..
	20	6	..	..	..	30	..	..	..	82	..	..	..
2	143	..	13	..	..	34	..	..	..	39	..	..	..
	163	..	16	..	..	36	..	..	..	38	..	..	..
	56	..	8	..	..	15	..	..	..	26	..	..	..
	29	..	11	..	..	23	..	..	..	29	..	..	..
3	192	..	..	32	..	..	..	44	..	..	..	34	..
	178	..	..	32	..	..	..	30	..	..	..	27	..
	23	..	..	28	..	..	..	26	..	..	..	20	..
	25	..	..	35	..	..	..	31	..	..	..	19	..
4	163	..	..	..	28	..	..	..	20	..	..	..	6
	200	..	..	..	26	..	..	..	17	..	..	..	0
	18	..	..	..	12	..	..	..	4	..	..	..	0
	72	..	..	..	30	..	..	..	27	..	..	..	12
5	137	9	..	..	..	17	..	..	..	31	..	..	..
	136	12	..	..	..	20	..	..	..	27	..	..	..
	131	8	..	..	..	16	..	..	..	20	..	..	..
	124	6	..	..	..	24	..	..	..	34	..	..	..
6	75	..	8	..	..	..	—	..	..	..	25	..	..
	77	..	8	..	..	..	—	..	..	..	27	..	..
	72	..	7	..	..	..	—	..	..	..	23	..	..
	86	..	10	..	..	..	—	..	..	..	30	..	..
	25	..	16	..	..	..	—	..	..	..	20	..	..
	36	..	8	..	..	..	16	..	..	..	10	..	..
	17	..	35	..	..	..	37	..	..	..	47	..	..
7	141	..	..	24	..	..	..	12	..	..	..	10	..
	131	..	..	13	..	..	..	17	..	..	..	14	..
	177	..	..	26	..	..	..	15	..	..	..	14	..
8	62	..	..	..	12	..	..	..	6	..	..	..	1
Average....		7	13	27	22	27	27	25	15	34	28	20	4

phenomena were noted, the figures being 89 per cent and 28.5 per cent. At 31°C. the maximum hatching of the dog strain larvae occurred on the second day, and the percentage fell from 82 to 50 per cent on the third day. Furthermore, the percentage of hatched larvae at 37°C. was highest for the first 24 hours, and decreased each day thereafter. These figures suggest that the absence of food is a factor, and that the higher temperatures increase the rate of development. This is probable in view of the fact that the metabolic rate of cold-blooded animals varies directly with temperature. Consequently, then, the explanation

follows that at 37°C. the eggs develop rapidly and the larvae when hatched are maintained in great activity so that, in the absence of food, they quickly die. This too accounts for the decrease in the number of larvae from the second to the third day at 31°C. It becomes necessary then to explain why the larvae at 21°C. were able to live after having hatched in the absence of food, especially when 82 per cent were hatched during the first day. In keeping with the same explanation, the reason suggested is that their metabolic rate was not accelerated by this temperature to the point of exhaustion. From the tables it is clear that a temperature of 37°C. is not favorable for the development of the eggs of either strain, and that the larvae which hatch are quickly activated to death.

In general these results support the findings of McCoy (1930) who studied the development of eggs to the infective larval stage at a variety of temperatures ranging from 12°C. to 42°C. Under the conditions of his experimentation 23°C. and 30°C. proved to be most favorable for development. It was felt, however, that the optimum temperature approached 30°C., while the present studies would indicate an optimum temperature approaching 21°C. These differences are easily understood in view of one important difference in the experimental method followed. In the present studies, the larvae were hatched in the absence of food, while in McCoy's work the larvae were cultured in bacterial growth. It seems entirely probable, from the nature of the data presented, that had the present studies been carried on under conditions of hatching in bacterial culture, a temperature of 30°C. would have proved as satisfactory as one of 21°C. Furthermore, data presented in Table 3 strongly support this expectation.

Experiments designed to test differences in development to the infective larval stage, showed in general the same striking differences between the two strains as noted above for hatching. The data affecting development to the infective larval stage have been combined for both strains in Table 3. Here it is seen that averages for a considerable number of experiments demonstrated a definitely higher rate of development of dog strain eggs. At 21°C., an average of 44 per cent of the eggs developed to infective larvae after ten days, while an average of only 5 per cent was obtained for the cat strain. Better developments were obtained at 31°C., the figure for the dog strain being 47 per cent, as opposed to 6 per cent for the cat strain. Comparing these figures with the percentage hatching at the same temperature, it is apparent that the ratio is reversed. This observation tends to indicate that a temperature of 31°C. is more favorable to the maintenance of life and normal development of the larvae, provided there is an abundant food supply.

While it is not possible to observe directly the numbers of hatched larvae which grow to the infective stage, a comparative rate of development for the larvae of the two strains can be ascertained by utilizing the results on percentage hatching and those on percentage development to the infective stage. In accordance with the methods followed,

TABLE 3.—*Percentage Development to Infective Larval Stage of Eggs of the Dog Strain and Cat Strain of A. caninum. Incubation for Ten Days in Cultures of Bacillus coli*

Experiment Number	Dog Strain Eggs				Cat Strain Eggs			
	Eggs Cultured	Larvae Recovered	Percentage Development		Eggs Cultured	Larvae Recovered	Percentage Development	
			at 21° C.	at 31° C.			at 21° C.	at 31° C.
1	7,000	2,600	37	..	900	32	4	..
	7,000	2,850	41	..	900	34	4	..
	7,000	2,000	30	..	900	10	1	..
	7,000	2,530	36	..	900	15	2	..
	7,000	2,790	40	..	900	12	1	..
	7,000	2,730	40	..	.....	...	..	..
2	16,400	6,300	..	40	3,260	90	..	3
	16,400	8,700	..	53	3,260	150	..	5
	16,400	2,270	..	13	3,260	110	..	3
3	10,000	3,500	..	35	1,840	186	..	10
	10,000	2,700	..	27	1,840	149	..	8
	10,000	2,816	..	28	1,840	124	..	7
4	4,000	1,755	..	44	400	22	..	5
	4,000	2,250	..	56	400	14	..	4
	4,000	2,360	..	59	400	15	..	4
5	2,480	1,350	..	54	5,140	45	..	1
	2,480	1,100	..	44	5,140	78	..	2
	2,480	1,109	..	45	5,140	61	..	1
6	1,480	880	..	60	3,900	400	..	10
	4,440	2,640	..	60	3,900	310	..	9
	4,440	2,880	..	60	3,900	225	..	5
	4,440	1,980	..	45	3,900	270	..	7
7	260	180	69	..	800	26	3	..
	390	250	64	..	800	90	11	..
	390	190	50	..	800	80	10	..
	390	120	30	..	800	70	9	..
8	3,000	1,670	..	55	600	5	..	1
	3,000	1,280	..	43	600	3	..	1
	3,000	1,960	..	65	600	2	..	1
9	.....	.....	..	..	3,050	150	..	5
	.....	.....	..	..	3,050	264	..	8
	.....	.....	..	..	3,050	170	..	5
	.....	.....	..	..	3,050	204	..	7
	.....	.....	..	..	3,050	291	..	9
	.....	.....	..	..	3,050	468	..	15
	.....	.....	..	..	3,050	360	..	12
	.....	.....	..	..	3,050	500	..	16
	.....	.....	..	..	3,050	220	..	7
	.....	.....	..	..	.....	.....	..	..
Average development.....			44	47				5 6

the difference between the percentage hatching and the percentage development to the infective stage represents the percentage of larvae surviving from the third to the tenth day of culturing. This figure has been calculated to be 49 per cent at 21°C. and 94 per cent at 31°C. for the larvae of the dog strain as compared to 17 per cent at 21°C. and 30 per cent at 31°C. for the larvae of the cat strain. This finding agrees very well with McCoy's (1930) observation that the best development of the eggs of the dogstrain was obtained at about 30°C.



## SUMMARY

The method of McCoy for obtaining hookworm eggs free from feces and growing the larvae in pure cultures of bacteria presented a means of comparing the viability and rate of development of the eggs and larvae of the two physiological strains of *Ancylostoma caninum*. For eggs of the dog strain, there was obtained a percentage hatching of 89 per cent at 21°C. and 50 per cent at 31°C. as compared to 28.5 per cent and 20 per cent respectively at similar temperatures for the eggs of the cat strain. Studies of the development of the eggs to the infective larval stage showed, for the dog strain, 44 per cent at 21°C. and 47 per cent at 31°C., and for the cat strain, 5 per cent at 21°C. and 6 per cent at 31°C. From these figures the percentage development of hatched larvae to the infective larval stage was computed. For larvae of the dog strain, this figure was 49 per cent at 21°C., and 94 per cent at 31°C., while for the cat strain, the figures were 17 per cent and 30 per cent respectively. These findings add to the number of experimentally demonstrated differences between the cat and dog strains of *A. caninum*.

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# STUDIES ON CILIATES FROM BERMUDA SEA URCHINS \*

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WITH INTRODUCTION AND NOTES BY

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## INTRODUCTION

Available records indicate that Hoffman (1871) was the first to call attention to the existence of ciliates in the digestive tract of European sea urchins, but he gave no descriptions. Maupas described *Cryptochilum echini* from the Mediterranean urchin, *Strongylocentrotus* (*Echinus*) *lividus* in 1883, and since then additions have been made by Di Mauro (1904), Andre (1910), Russo (1914) and Hentschel (1924).

It appears that Jacobs (1914) was the first to record the presence of ciliates in American sea urchins. He mentions four kinds which he found in *Diadema setosum* and studied physiologically at the Tortugas Islands, but he gives no names, referring to them by the letters, A, B, C, and D. Bray (1925) observed ciliates in *Toxopneustes variegatus* at Bermuda in 1919 and in the same host at Beaufort, N. C., in 1924.

In the summer of 1923 Miss Ruth Jane Ball (now Mrs. Walter T. Biggar), then assistant professor of zoology at the University of Vermont, made a study of the ciliates of the sea urchins of Bermuda. Mrs. Biggar presented an account of her studies at the Washington meeting of the American Society of Zoologists, in December, 1924, suggesting new names for five species. In the abstract (Ball, 1924) which was published, however, no new names were included. Subsequent preoccupation has prevented Mrs. Biggar from adding to her studies or preparing her material for publication.

Meanwhile, Dr. J. E. Lynch has begun an investigation of the ciliates of the sea urchins of the Pacific coast and has already published two papers (Lynch, 1929, 1930). In 1930, further studies were begun on the ciliates of the Bermuda sea urchins by Dr. Miriam Scott Lucas, and on those from the urchins of the coast of Maine by Mr. Philip Powers.

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\* Contributions from the Bermuda Biological Station for Research. No. 166.

At the request of Dr. E. L. Mark, Director of the Bermuda Biological Station, the writer has undertaken to prepare for publication a short paper giving the main results obtained by Mrs. Biggar. In the preparation of this paper the writer wishes to acknowledge, with thanks, the advantages he has had of personal conferences and correspondence with Dr. Lucas, Dr. Lynch and Mr. Powers, and an opportunity to examine some of their material. With these aids, and especially with the help of slides prepared by Dr. Lucas, and material sent from Bermuda by Mr. J. Kenneth Donahue, an attempt has been made to give a taxonomic status to four of the ciliates, the drawings and descriptions of which are found in Mrs. Biggar's notes. The fifth species mentioned by Mrs. Biggar was not characterized in sufficient detail to be named.

In arranging Mrs. Biggar's material, her original drawings have been used together with her descriptive notes. It should be mentioned that Mrs. Biggar's studies were limited largely to the living animals, hence some details are insufficiently indicated in the drawings. Likewise, her measurements are somewhat different from those obtained from preserved animals. In her notes, Mrs. Biggar assigned all four species to new genera, but since she did not mention any names in her published abstract, the writer has found it advisable to assign the four new species to existing genera. However, Mrs. Biggar's new species names are used. The notes and discussions added by the writer are enclosed in brackets. [D. H. W.]

#### DESCRIPTION OF SPECIES

Genus *METOPUS* Claparède et Lachmann 1858-59

*Metopus circumlabens* n. sp. (Figs. 1 and 1a)

Morphology: Elongate-oval in outline, rounded anteriorly and tapering posteriorly in such a manner as to suggest an interrogation mark; region anterior to oral groove hook-like and cristiform; body flattened, especially anteriorly; animal highly flexible, particularly the anterior hook-like flap or crest. Peristome a deep furrow extending from a notch in the upper left hand margin (right in Fig. 1) in a sweeping curve parallel to the anterior border, narrowing into a short pharynx near the right margin and near the transverse axis; numerous food vacuoles confined to the posterior part of the body; anal opening at posterior extremity (Fig. 1a; egestion shown in Fig. 1). Cilia distributed over the body in oblique rows, somewhat longer anteriorly, especially differentiated along the lower border of the peristome [zone of membranelles]; a tuft of about twelve much longer cilia at the posterior end; a flagellum [undulating membrane?] protruding from the lower border of the pharynx. Three contractile

vacuoles, one near the posterior end, one to the right of the gullet and the third opposite the second on the left side of the animal. Macronucleus oval in profile, immediately posterior to the peristome [accompanied by a single micronucleus]. Size: 170 by 100 $\mu$ . Swims usually in a circular path.

Hosts: Found in large numbers in *Diadema setosum* and in *Echinometris subangularis*. Corresponds to species "B" of Jacobs.

[Mrs. Biggar had placed this new species in a new genus, but its obvious relationships to the species of *Metopus*, as recently defined by Kahl (1927), make it seem more logical to place it in the latter genus.]

Genus *CRYPTOCHILUM* Maupas 1883

*Cryptochilum bermudensis* n. sp. (Fig. 2)

Morphology: Shaped like a scoop; anterior two-thirds of body thin and leaf-like; posterior region thickened, terminating in a rudder-like style. Protoplasm colorless, transparent, highly alveolar in structure. Oral aperture on left border [as drawn] in the posterior third of the animal; food vacuoles confined to the posterior third of the body; anal aperture not observed. Cilia arranged in rows, indicated by 35 to 40 longitudinal striations on a side; more conspicuous on anterior margin, where they appear to be inserted on conical elevations; also more noticeable in the region of the cytostome; a group of longer cilia on the caudal style. Contractile vacuole single, surrounded by several small vacuoles, located near the posterior end. Macronucleus single, rounded, near the middle of the body; micronucleus single, close to the macronucleus, often containing three refractive granules. Size 130 by 75 $\mu$ . Movement very moderate as to speed, often in a circular path, rotating on its longitudinal axis.

Reproduction: Several seen in transverse fission.

Hosts: Abundant in all of 50 specimens of *Toxopneustes variegatus* examined. Probably corresponds to species "D" of Jacobs.

*Cryptochilum echinometris* n. sp. (Figs. 3 and 4)

Morphology: Has scoop-like form as in the preceding species, but is smaller, relatively narrower, with anterior end more symmetrically rounded, and has fewer longitudinal striations. Protoplasm transparent but in the anterior region densely granular instead of alveolar. Oral aperture farther forward than in *C. bermudensis*, but posterior to the middle of the body; food vacuoles confined to posterior region. Contractile vacuole single, near posterior end. Rounded macronucleus near center of body accompanied by one micronucleus. Size 73 by 26 $\mu$ .

Host: Found only in *Echinometris subangularis*.



[The two preceding species were assigned by Mrs. Biggar to a new genus. However, they appear to have such close affinities to *Cryptochilum echini* Maupas that they are now assigned to the same genus. Of the two, *C. echinometris* is the more nearly related to *C. echini*, but it has a somewhat different contour and the cytostome is considerably behind the middle of the body, whereas that of *C. echini* is at, or slightly anterior to, the middle. The nucleus of *C. echinometris* is also located more posteriorly. *C. bermudensis* (Fig. 2) is still further removed from the type species, showing an increase in size and in relative width, and having a still more posterior position for the cytostome. That these species are related to *Cryptochilum echini* is indicated by the report of Bray (1925), who states that he found "*Cryptochilum echini*" in all but one of 22 specimens of *Toxopneustes variegatus* secured at Beaufort, N. C., and that he saw a form resembling *C. echini*, but possibly a new species, in the same urchin collected in Bermuda.]

Genus ANOPHRYS Cohn 1866

*Anophris elongata* n. sp. (Figs. 5 and 6)

Morphology: Body cigar-shaped, anterior end somewhat pointed, posterior end more rounded, anterior third narrower, longitudinally striated, very transparent and flexible (Fig. 5); posterior two-thirds contains many refractive bodies. Mouth obscure, ingestion not observed; anal aperture not determined. Cilia in longitudinal rows, more evident in the anterior third of body; a ciliated membrane extends out from the anterior third as far back as the oral indentation [not shown in drawings]. Contractile vacuole single, located close to the posterior extremity. Macronucleus near the center of the body. [A micronucleus close beside it.] Size: 166 by 33 $\mu$ . Movement very rapid, darting through the water in a straight or spiral path.

Reproduction: Several specimens seen in transverse fission.

Hosts: *Toxopneustes variegatus* and *Echinometris subangularis*. Probably corresponds to species "C" of Jacobs.

[The description and drawings of this ciliate as recorded by Mrs. Biggar are somewhat incomplete. Specimens on a slide prepared by Dr. Lucas show that the cilia are quite long, arranged in about 16 to 18 longitudinal rows and that there is a long caudal cilium or filament in addition. The oral membrane mentioned by Mrs. Biggar is not shown on her drawings, but appears to extend from the anterior end to the oral vestibule and along the right side of this cavity. It appears to consist of long cilia, set close together, gradually increasing in length backward to the oral depression and somewhat shorter within that cavity. In life they would probably simulate a membrane.

The taxonomic status of this species is open to question. Mrs. Biggar proposed to place it in a new genus but it should probably be placed in an existing genus. It seems to be related to *Lembus*, but species of that genus have two oral membranes (Hoare, 1927), while only one has been made out for the present species. A single membrane is said to characterize *Cyclidium*, but the species under discussion lacks several of the characters of that genus, such as the existence of an extensive peristome. The genus *Anophrys* was established by Cohn (1866) for *A. sarcophaga*, which resembles the present species somewhat, but the form referred to by Rees (1884) as *A. sarcophaga*, shows a much closer resemblance. Di Mauro (1904) has described *Anophrys echini* from *Strongylocentrotus lividus* and *Sphaerechinus granularis*, but he states that it lacks a caudal filament. However, such a filament is easily overlooked, and his figure shows a membrane-like row of cilia along the right side of the peristome. Russo (1914) suggests that Di Mauro's species might belong in the genus *Lembus*, apparently overlooking the fact that *Lembus* has two oral membranes. The species found by Mrs. Biggar is larger, relatively thinner, and more definitely narrowed for the anterior third of the body, as compared with Di Mauro's *Anophrys echini*, hence her new species name, *elongata*, is employed.

Mrs. Biggar also found and partially described a ciliate which she identified as the one designated "A" by Jacobs. It is somewhat more than twice as long as broad, much flattened, rounded at both ends, with a posterior contractile vacuole and an oral cleft near the anterior end. The macronucleus is anterior to the middle and the region anterior to it is filled with refringent granules. Size: 35 by 16 $\mu$ . From the partial description and sketches furnished by Mrs. Biggar, one discerns a resemblance to the form called *Cryptochilum boreale* by Hentschel (1924), but it is much smaller. It is probable that neither the present species nor that of Hentschel belongs to the genus *Cryptochilum*, but since no name for it was given in Mrs. Biggar's notes, and since its characters are insufficiently determined, it will be left for future workers to study and give an appropriate taxonomic status.

In going over material obtained from the digestive tract of sea urchins from the coastal waters of North America and Bermuda, one is impressed with the considerable number of ciliates which are found as endozoic associates of these hosts, and also with the fact that many of them are so closely related to free-living species. The entire situation promises to provide very interesting data bearing on the question of the origin of the endozoic habit of such ciliates.]



BIGGAR—CILIATES FROM SEA URCHINS

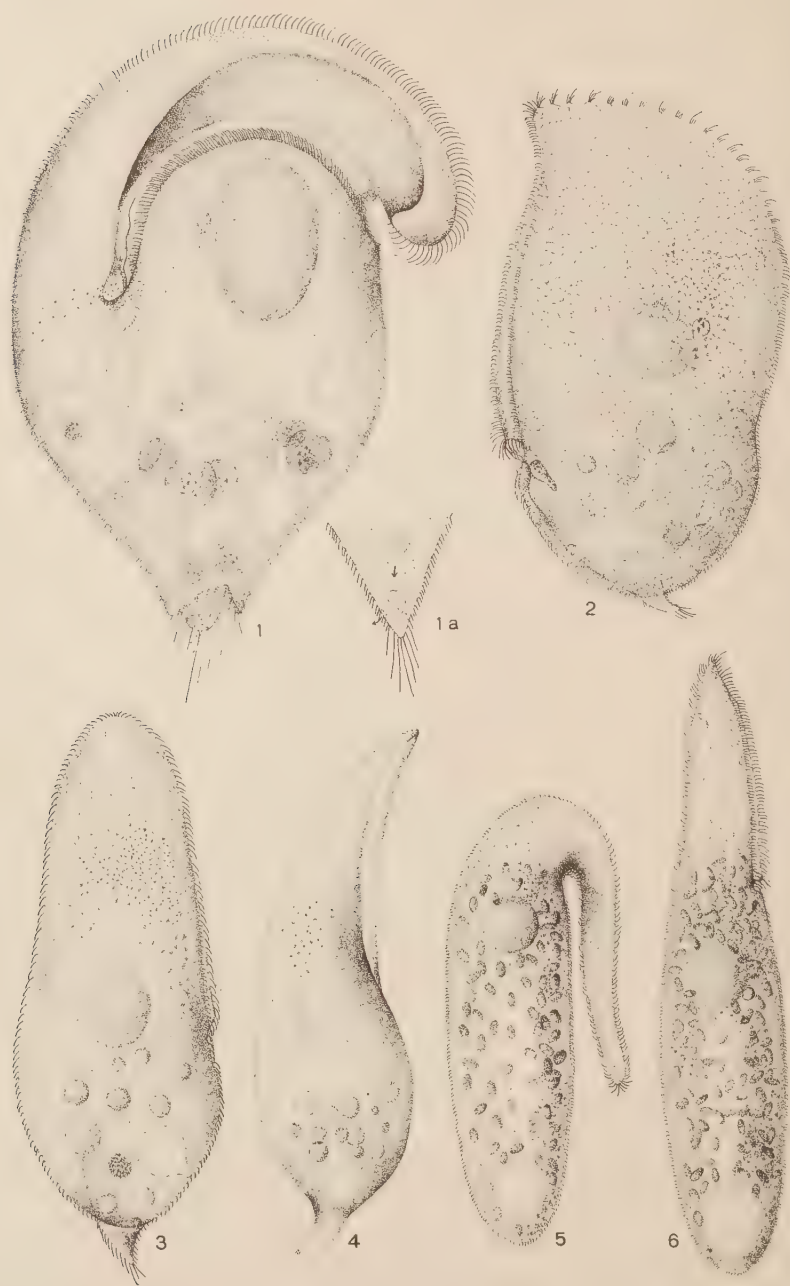


PLATE XXIII



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## EXPLANATION OF PLATE XXIII

All figures, except 3 and 4, are from drawings made originally at a magnification of 1000 diameters; figures 3 and 4 are from drawings made at  $\times 2000$ ; all are reduced in printing to about one-half the original diameter. Figures 1 to 6 are from drawings made by Mrs. Biggar from fresh material. Figure 1a was copied from an original drawing by Mrs. Biggar.

Fig. 1.—*Metopus circumlabens*, ventral view; posterior end invaginated, as during egestion.

Fig. 1a.—*Metopus circumlabens*, ventral view; posterior end under normal conditions, as arrows show path of movement of food vacuoles to anal aperture.

Fig. 2.—*Cryptochilum bermudensis*, side view, showing general features of organization.

Fig. 3.—*Cryptochilum echinometris*, side view, showing general morphology.

Fig. 4.—*C. echinometris*, edge view, showing scoop-like profile.

Fig. 5.—*Anophrys elongata*, edge view, anterior end bent backward, showing flexibility of this region.

Fig. 6.—*A. elongata*, side view. Showing general morphology.

# ON THE ANATOMY AND SYSTEMATIC POSITION OF THE CAUSATIVE AGENT OF SO-CALLED SALMON POISONING

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Donham (1925) and later Donham, Simms and Müller (1926) described a fatal disease of dogs on the Pacific Coast of North America following the ingestion of uncooked salmon. These authors emphasized the constant association of the disease with a small intestinal trematode which they considered as the causative agent. The metacercariae of this parasite were found in the muscles and various organs of several species of salmonid fishes of the genera *Salmo* and *Oncorhynchus* caught in North American waters.

The trematode was identified the following year (1926) by Chapin who considered it to be a new species of the family Heterophyidae, and named it *Nanophyes salmincola*. The generic name proved however preoccupied and was emended by Chapin (1927) to *Nanophyetus*.\*

Subsequently *Nanophyetus salmincola* was found by Cram (1926) in coyote (*Canis lestes*), raccoon (*Procyon psora pacifica*) and a lynx (*Lynx fasciatus fasciatus*), but it did not appear to be as pathogenic for these animals as for the dog.

The only complete description of this trematode accompanied by a diagram of the anatomical structure is that given by Chapin (1926). While studying the original material † for the revision of Heterophyidae, I found some discrepancies between its anatomy and the previous description and illustration. It appears that *Nanophyetus salmincola* has no genital sucker or similar organ and no seminal receptacle and has a conspicuous cirrus pouch. The eggs proved to be much smaller than the suckers and not the contrary, as is shown on Chapin's illustration.

Skrjabin and Podjapolskaja (1931) described *Nanophyetus schikhobalowi* from the aborigenes of East Siberia. This species was

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\* Some confusion exists with regard to the author's name for this genus. I have to thank Mr. Hall for permission to publish the following explanation: "under the impression that Chapin's name *Nanophyetus* had been published I referred to it in my paper which appeared early in 1927, a few months before Chapin's proposal of the name appeared. Consequently the name *Nanophyetus* should be cited as *Nanophyetus* Chapin in Hall, 1927."

† I have to thank Dr. A. Hassall of the Bureau of Animal Industry, Washington, D. C., for his courtesy in sending me several specimens of *Nanophyetus salmincola* obtained experimentally.

distinguished from *Nanophyetus salmincola* by the smaller size of the eggs as compared with the original description of Chapin and in some minor features, which can be attributed to age or the influence of fixation, but have no specific value.

A re-examination of the American material at my disposal showed that anatomical details in *Nanophyetus salmincola* are more variable than is mentioned by both Chapin and Skrjabin and Podjapolskaja, and that *Nanophyetus schikhobalowi* is identical with *N. salmincola*. The substantial difference between the description of Skrjabin and Podjapolskaja and our data consists in that in *N. schikhobalowi* no cirrus pouch and no esophagus were observed, while in *N. salmincola* both are conspicuous. In my opinion Skrjabin and Podjapolskaja's description is incomplete, for it is hardly possible, that two species similar in every other detail should differ so fundamentally in the structure of these organs.

The following is the description of *Nanophyetus salmincola*, obtained in America from experimental dogs\*:

The worms are pyriform, slightly flattened dorsoventrally, 0.8 to 1.1 mm. long and 0.3 to 0.5 mm. wide (0.5 by 0.3 to 0.4 mm.). The oral sucker is 0.15 to 0.18 mm. in diameter (0.08 to 0.1 by 0.12 mm.) and opens subventrally. It is followed by a pharynx 0.06 mm. long (0.04 to 0.07 mm.) which is continued into an esophagus 0.06 to 0.07 mm. long. The ceca are much wider than the esophagus. They extend up to the level of the posterior part of the testes and lie between the testes and dorsal surface of the body. They present the shape of a horseshoe with a wide arc and narrower base. The ventral sucker is 0.12 to 0.13 mm. in diameter (0.11 to 0.12 mm.) and lies in the middle of the ventral surface of the body or a little in front.

Two large oval testes 0.2 to 0.3 mm. long (0.17 to 0.2 mm.) are situated symmetrically at the sides in the posterior half of the body. They lie obliquely to the long axis of the body. The cirrus pouch is situated behind the ventral sucker and to the left of the ovary. It is relatively large, up to 0.2 mm. long, has thin walls and contains a pars prostatica and a large seminal vesicle divided in two parts by a constriction.

The ovary is almost round, 0.07 to 0.11 mm. in diameter (0.04 to 0.08 mm.) and is situated on the right side in the intercecal space. A seminal receptacle is not in evidence. Laurer's canal opens dorsally on the right side at the level of the esophagus. The uterus consists of two coils directed posteriad which, when seen from the side, form a W. It opens into the tube-like genital sinus which is situated near

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\*The numerical data of Skrjabin and Podjapolskaja, which do not conform with ours are given in brackets.

the posterior edge of the ventral sucker. The whole course of the uterus lies in the sagittal plane of the body and therefore in mounted specimens it appears as a short row of eggs, 10 to 15 in number, lying between the testes. The eggs are oval, 64 to 80 $\mu$  long and 34 to 50 $\mu$  wide measured in mounted specimens. The vitellaria consist of irregular follicles scattered under the dorsal surface of the body, except in the anterior region which is free. They are often very thick and obscure other anatomical details in mounted specimens. The excretory vesicle is not well seen in our material but seems to be sac-shaped and is situated behind and between the testes.

There exist some discrepancies with regard to the systematic position of *Nanophyetus salmincola*. As already mentioned Chapin ascribed it to the family Heterophyidae. In an earlier paper (1929) the present author emphasized, on the basis of re-examination of the original material, that *Nanophyetus* does not belong to this family. However, Skrjabin and Podjapolskaja (1931) accepted Chapin's opinion. The most characteristic features of the Heterophyidae are: absence of a true cirrus pouch, presence of a large seminal receptacle and of peculiar, for the most part armed, genital papillae or gonotyls. All these features are absent from *Nanophyetus salmincola*. The author is of the opinion that the genus *Nanophyetus* corresponds exactly to the genus *Troglootrema* Odhner 1914, of the family Troglo-trematidae Braun 1915 and should be regarded as a synonym. *Nanophyetus salmincola* should therefore be renamed *Troglootrema salmincola* (Chapin, 1926). Another species of this genus, *T. acutum* (Leuckart), has been recently redescribed and illustrated by Baer (1931). *Macroorchis spinulosus* Goto in Aado, 1919 (see Dollfus, 1925:197), is probably a third species, but this can only be determined through a restudy of this species which is insufficiently described. The two certain species may be distinguished by the following differences:

	<i>T. acutum</i>	<i>T. salmincola</i>
Ceca.....	Sinuous and extend beyond the testes	Smooth and do not reach the posterior extremities of the testes
Uterus.....	Makes longitudinal and transverse loops	Loops only in the sagittal plane
Localization.....	Frontal sinuses of polecats.....	Intestine of carnivora and man

The description of the genus *Troglootrema* was given by Odhner (1914) and was based on contracted specimens of *T. acutum*. If the above-mentioned description of *T. salmincola* and the description of *T. acutum* given by Baer (1931) be taken into consideration, the generic diagnosis of *Troglootrema* can be emended as follows:

## TROGLOTREMATIDAE

Both suckers well developed. Testes and ovary with smooth outlines. Cirrus pouch well developed, containing double seminal vesicle and pars prostatica. Seminal receptacle very small or absent. Uterus confined within the intercecal space. Vitellaria very well developed, under the dorsal surface of the body. Both male and female organs open in a deep genital sinus devoid of any additional structures and located behind the ventral sucker. Free in the intestine or frontal cavities of mammals.

Type species: *T. acutum* Leuckart, 1842.

Besides the genus *Troglorema* three more genera belong to the family Troglorematidae: *Paragonimus* Braun, 1899, *Renicola* Cohn, 1904, and *Collyriclum* Kossack, 1911.\* They may be distinguished according to the following key:

A. Living in crypts or cysts; ovary lobate;

1 testes lobate:

a, uterus occupies almost entire body, forms

uterine sac .....RENICOLA †

b, uterus occupies a small space in center of

body, does not form uterine sac.....PARAGONIMUS

2 testes with smooth outlines.....COLLYRICLUM

B. Living free in open cavities or intestine of

mammals; neither ovary nor testes lobate.....TROGLOREMA

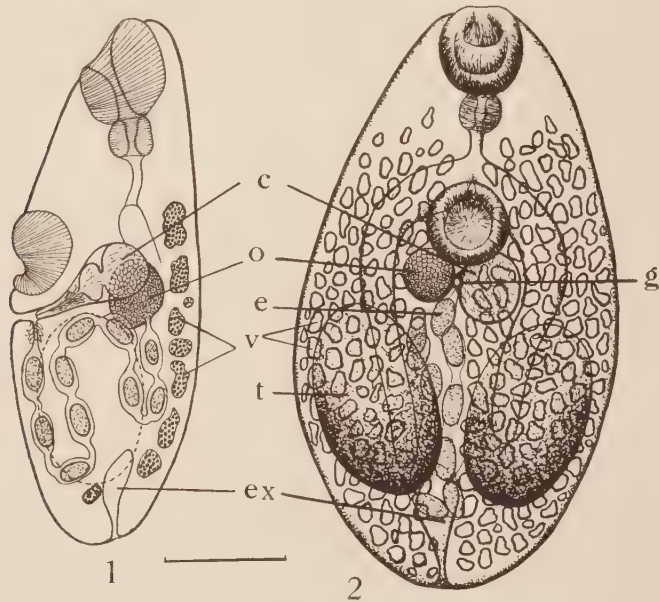
The inclusion of the causative agent of the so-called salmon poisoning in the genus *Troglorema* is important not only from the zoological standpoint but for human hygiene, for this parasite has been found in man. Its inclusion in the Heterophyidae, as suggested by previous authors, would indicate that the parasite is comparatively little harmful. In view of its inclusion in the genus *Troglorema* its pathological significance, though still little known, may a priori be considered as serious. As a matter of fact, for dogs *Troglorema salmincola* is a very dangerous parasite. Hoepli (1926) described severe histological changes of the intestinal mucosa in dogs caused by this parasite. Donham, Simms and Müller (1926) give, inter alia, the following details on the disease produced experimentally in dogs by feeding them on salmon infected with metacercariae of *Troglorema salmincola*:

\* Odhner (1914) and Jegen (1917) also ascribe the genera *Pholeter* Odhner, 1914, and *Brandesia* Stossich, 1899, to Troglorematidae. However, I regard the genus *Pholeter* as synonym of the genus *Collyriclum* and I agree with Poche (1926) in excluding the genus *Brandesia* Stossich from Troglorematidae.

† Most of the species of the genus *Renicola* are insufficiently described. The generic characters of *Renicola* used in this key are based on the description of *R. glandoloba* Witenberg, 1929a.



"The symptoms do not develop until 7 to 10 days after the dog has eaten the fish . . . the onset is very sudden, the temperature rises to 105 to 107 F. . . . symptoms usually last for 24 to 48 hours after which the temperature is gradually lowered. At this time the diarrhea develops which is blood-tinged at first and later is practically all blood. . . . In about 6 to 8 days the temperature usually becomes subnormal and death occurs 24 to 48 hours later. An occasional case recovers . . . 1 gram of infected salmon may sometimes produce death. . . ."



EXPLANATION FOR TEXT FIGURE

*Troglotrema salmincola*. 1. Sagittal section (semidiagrammatic); 2. ventral view. Scale equals 0.2 mm. *c*, cirrus pouch; *e*, egg; *ex*, excretory bladder; *g*, genital pore; *o*, ovary; *t*, testis; *v*, vitellaria.

One may suppose that in man the parasite produces similarly serious symptoms, but so far nothing is known about them.

It is noteworthy that the second species of this same genus, *T. acutum*, produces serious pathological changes in the frontal sinuses of polecats. According to Olt (1929) the parasites are "in direct contact with the blood vessels and produce decalcification of the wall of the sinus. The wall becomes membranous at the site where the parasites fix themselves and this results in the formation of openings with irregular edges, visible on the prepared skulls" (Translated from Baer, 1931).

It may be mentioned here, that some other species of the same family are known as injurious parasites of man (*Paragonimus ringeri*) and poultry (*Collyriclum faba*).

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## LIFE HISTORY OF THE NORTH AMERICAN LUNG FLUKE OF MAMMALS\*

DONALD J. AMEEL

On July 29, 1931, a collection of snails of the species *Pomatiopsis lapidaria* taken near Ann Arbor, Michigan, yielded several individuals shedding microcercous cercariae closely resembling the cercaria of the human lung fluke, *Paragonimus*. Numerous cercariae were placed in a dish with parasite-free crayfishes. They soon disappeared from sight and an examination of the ventral side of a crayfish revealed numerous cercariae penetrating through the thin chitin of the tail at the union of the segments. About twenty hours after exposure, eleven cercariae were found in the heart muscles of one of these crayfishes. Later examination of other crayfishes of the same lot revealed similar infections. The cercariae did not encyst immediately, but individuals were found one to two days after infection completely enclosed in a very thin membrane. After a four weeks sojourn in the crayfishes, encysted metacercariae were recovered that agreed in every respect with proven *Paragonimus* metacercariae found in naturally infected crayfishes of the same region.

Although this cercaria differs in certain details from Kobayashi's figures and descriptions of the Asiatic lung fluke, there are many points of similarity. A comparison of the work of Kobayashi (1921) and of Faust (1929) on the same species shows several discrepancies. My material agrees much more closely with Kobayashi's figures than with Faust's. Measurements in microns were made of fifteen specimens killed in 5% formalin and slightly flattened under a number one cover slip. The averages are as follows: total length, 178; width, 93; tail, 15 by 14 (often perfectly spherical); oral sucker, 48 (usually slightly elongated anteriorly); stylet, 39 by 7; acetabulum, 47 by 34. The cercaria is completely covered with spines of about equal length which are most conspicuous on the posterior portions of the body and tail. The group of large spines located on the tip of the tail is particularly conspicuous, for the remainder of the tail is sparsely covered with minute spines. Penetration glands surround the lateral and anterior edges of the acetabulum but usually do not extend beyond its posterior border. These can be differentiated into two types by their affinities for neutral red: a lateral group of four large cells on each side of the body which have a great affinity for this stain, and a median mass of six smaller cells which stain poorly. Four large ducts leave each group of

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\* Contribution from the Department of Zoology, University of Michigan.

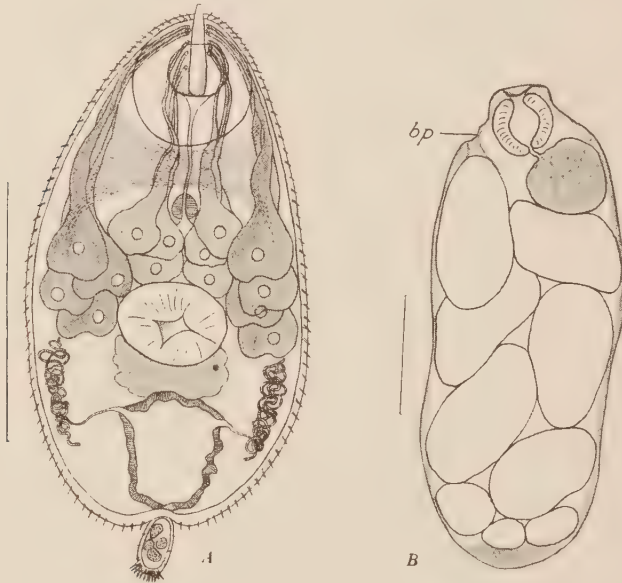
lateral cells, proceed along the edge of the cercaria, penetrate the oral sucker, and open on each side of the stylet. Six ducts of about the same size as the lateral ones arise from the central cells, proceed forward in the mid-region of the cercaria in two groups of three each and penetrate the oral sucker, opening three on each side of the stylet just posterior to the openings of the lateral ducts. A long prepharynx is present, and a pharynx is located about midway between the oral sucker and the acetabulum. The remainder of the digestive tract is still undifferentiated. The brain is a conspicuous butterfly-shaped mass situated between the oral sucker and the pharynx, extending to the ducts of the lateral penetration glands. The genital primordium is represented by a mass of cells posterior to the acetabulum. A median, thick-walled excretory bladder about as broad as the acetabulum occupies the region between the latter and the posterior end of the body.

One of the most striking differences between this cercaria and Kobayashi's is the character of the penetration glands and their ducts. The only ducts described and figured by him lie in the lateral fields and arise from groups of fairly small cells of unknown number. Another feature of Kobayashi's cercaria is a group of cells larger than the penetration glands, which Kobayashi calls "gland-like cells." These cells are located along the median ventral part of the body and fill up the regions anterior and posterior to the acetabulum. Kobayashi did not note ducts arising from these cells. Judging from Faust's figure and this material, these may prove to be penetration glands. The genital primordium of Kobayashi's cercaria is less than twice that of the acetabulum while this cercaria has an oral sucker with a diameter not much greater than that of the acetabulum. In other respects, the cercariae resemble one another closely. However, no precise comparison can be made, for Kobayashi apparently worked with preserved material, while the present study was made almost exclusively on living material with the aid of *intra vitam* stains.

The cercaria described by Faust presents far greater differences from my material than does that described by Kobayashi. Faust pictured two types of penetration glands: two median groups of five large cells each, extending almost to the posterior end of the body; and two distinctly different groups, each consisting of four considerably smaller cells located between the larger cells and the margin of the body. All the ducts from these cells are located close to the median region. However, the most noteworthy differences are the location of the pharynx, which is shown immediately adjacent to the oral sucker, and the presence of pharyngeal glands with ducts opening into the pharyngeal region. The genital primordium is even more extensive than that shown in Kobayashi's figures. Faust says that the cercariae "swim around in the water." These cercariae are notable in their

inability to swim. They generally creep along a surface like a leech or, if the water is agitated sufficiently to cause them to lose their hold, they float, executing the same type of movement.

The rediae are located in the liver of the snail host. Their shape is that of an elongated ellipsoid, devoid of appendages. The cuticula is thrown into minute rugae over most of the body. The mouth is terminal, opening immediately into a large, spherical, muscular pharynx. A short esophagus leads into a short, usually spherical gut which is often smaller than the pharynx. The gut wall consists of a single layer of large, clear cells. Amber colored particles derived from the



Textfigure A.—Camera lucida drawing of a cercaria. Scale, 0.10 mm.

Textfigure B.—Camera lucida drawing of a redia, *bp*, birth pore. Scale, 0.10 mm.

tissues of the snail fill the lumen of the gut. The entire digestive tract is generally confined to the anterior third or fourth of the body. The remainder of the body cavity of the redia is filled with cercariae in various stages of development. A birth pore is located in the margin of the body adjacent to the pharynx. Germ balls lie in the posterior portion of the body. Average measurements of nineteen rediae slightly flattened under a number one cover slip are: length, 564; width, 226; pharynx, 76; gut, 70 $\mu$ . The redia described by Kobayashi has a long, fairly narrow gut extending posteriorly to the middle of the body. Kobayashi does not mention a birth pore.



The differences represented by the figures of the three cercariae under discussion, if valid, together with differences in the rediae, are sufficient to distinguish at least two species of lung fluke, an Asiatic and a North American, and the differences in the descriptions given by Kobayashi and Faust may indicate two species of the Asiatic lung fluke. However, a more thorough study of these cercariae is necessary before this species question can be definitely settled.

Metacercariae found in local crayfishes resemble those described and figured by Kobayashi and Faust. However, their location in the tissues of the Asiatic and North American hosts is decidedly different. Metacercariae in all the crayfishes I have examined were usually present in the heart tissue and occasionally in adjacent tissues but never in the gills or body muscles which are the parts commonly infected in the Asiatic crabs and crayfishes. This may prove to be another character for the differentiation of species. I have found metacercariae in *Cambarus propinquus*, *C. robustus*, *C. virilis*, *C. diogenes*, and *C. rusticus*, and have raised them experimentally in *C. immunis*. There is little doubt that they will develop in any species of crayfish exposed to infection.

Though dogs, cats and swine have been found infected with this worm, the mink has been proved by Wallace (1931) to be the true definitive host. Racoons eat large quantities of crayfishes but I have definitely proved by experiment as well as by examination of 308 carcasses that they are immune to infection.

Normally the definitive host becomes infected through the eating of crayfishes bearing the metacercariae, but it is also possible for the transmission of very small worms from one definitive host to another. Yokogawa and Suyemori (1920) experimented with this type of transmission but for some reason had no success. However, they cite the work of Kawamura and Ando, both of whom report positive results. Neither of these papers is available.

White rats and domestic cats were used in the following experiments. For some reason, as yet unexplained, worms from one to one and a half millimeters long remain in the pleural and abdominal cavities of white rats for months without penetrating the lungs. Such worms even after one and one-half months still retain their stylets. No stylets were found in worms older than these. Twelve 185-day old worms from the pleural cavities of several white rats were fed to a cat known to be previously uninfected. Seven weeks later, six adult worms were recovered from the lungs. Experiments involving worms after shorter periods of time in pleural and abdominal cavities of white rats were equally successful. However, negative results were secured by feeding twenty-four worms (two to three millimeters long), obtained from the

lungs. It has not been possible to determine that these worms linger in the body cavities of minks as they do in rats; nevertheless, the above experiments are proof enough that worms which have not entered the lungs are infective if eaten by another host. Thus, a mink during the first few weeks of infection is probably just as effective a carrier as a crayfish. I have collected information from trappers who have seen dogs and cats eat minks, and I have also induced cats to eat them. Two sufficiently starved laboratory cats willingly ate crayfishes, definitely proving that they may also become infected directly by the eating of infected crayfishes. I have no information on the eating of crayfishes by dogs.

I wish here to express my grateful appreciation to Professor George R. La Rue, under whom this investigation is being undertaken. I am also indebted to Dr. Helen F. Price for aid in the collection of host material.

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THE APPEARANCE AND SIGNIFICANCE OF THE  
UNFERTILIZED EGGS OF *ASCARIS*  
*LUMBRICOIDES* (LINN) \*

G. F. OTTO

Muira and Nishiuchi as early as 1902 gave a rather comprehensive discussion of the appearance and occurrence of the unfertilized egg of *Ascaris lumbricoides*. Despite the fact that these authors as well as most subsequent texts in parasitology have figured the unfertilized eggs they are not generally recognized by laboratory technicians making fecal diagnoses. In fact many parasitologists seem to consider them only of passing interest. The above authors also described the asymmetrical, often triangular type of egg, but it has never, to my knowledge, been figured and consequently is rarely recognized by anyone doing routine fecal examinations.

It is scarcely necessary to point out that when unfertilized eggs alone appear in the feces their recognition is important. With the development of quantitative methods of fecal diagnosis, whereby an attempt is made to estimate the number of worms present by counting the eggs found in a measured portion of the stool, the recognition of these eggs when they appear mixed with the fertilized eggs also becomes important.

Though the commoner unfertilized eggs have been described and often figured it seems worthwhile to review and describe them here. The figures and descriptions presented in this paper are principally from eggs observed in the N/10 (0.04 per cent) sodium hydroxide of the Stoll dilution egg counting preparation. Some eggs, however, were studied in simple saline-feces smears and found to be essentially the same as those in the N/10 sodium hydroxide. Figures 3, 4, 5 and 6 represent the commoner forms of unfertilized ascaris eggs. The albuminous outer coat (alb.) may or may not be present on eggs taken from the uterus (Wharton, 1915), found in the simple feces smear or seen in N/10 sodium hydroxide. The sodium hydroxide acts as a slow solvent of the albuminous coat distending it during that process. It is, therefore, not uncommon to find the unfertilized ascaris eggs in this medium having what appears to be an irregular wide, either transparent or bile stained, halo around the shell. This is well illustrated in figures 5 and 8 while figure 3 shows the albuminous coat distended slightly beyond normal. This is much less frequent in the normal fertilized eggs.

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\* From the Department of Helminthology of the School of Hygiene and Public Health of the Johns Hopkins University.

The writer is indebted to Dr. W. W. Cort for helpful criticisms and to Dr. John Stumberg for most of the photomicrographs.

No nucleus is apparent in the unfertilized eggs and the contents of the shell are never organized but rather are disintegrated and appear as finely granular or as larger globules of fat or yoke. These contents may be uniformly distributed or collected at one end or one side of the egg.

Muira and Nishiuchi (1902) dealing with these unfertilized eggs and Foster (1914) considering the unusually large fertilized eggs sometimes seen have pointed out that the diameters of all the different types of eggs of *A. lumbricoides* are essentially the same, the variation in size being primarily in length. The first authors report the average diameter of two groups of unfertilized eggs as 0.040 and 0.050 mm., the maximum and minimum diameters in the first group being 0.037 and 0.044 mm. The average lengths for these same groups were 0.076 and 0.0809 mm., the limits in the first group being 0.067 and 0.086. Foster considering the abnormally large fertilized eggs also found that the diameter averaged about 0.050 mm. The writer measured 30 unfertilized eggs from 20 different stools and found the maximum, average, and minimum diameters to be 0.041, 0.048, and 0.049 mm. and the lengths 0.066, 0.077 and 0.106 mm. without the outer albuminous layer of the shell. The widths are well within the range of normal fertilized eggs.

While the eggs shown in figures 3, 4, 5 and 6 are by far the most common type of unfertilized ascaris eggs seen there are a number of eggs which are so atypically shaped as to be passed unnoticed except by the most practiced observer. Figures 7, 8, 9 and 10 are representative of some of these types. It is not unusual to find these eggs alone in stools having a low egg count. Only one or two, or occasionally a larger percentage, may be also found per slide\* in heavy infestations. Except for shape their appearance is similar to that of the more typical unfertilized eggs. The shortest axis of these eggs is often greater than the diameter of the fertilized or of the more common unfertilized eggs. The longest axis, however, is rarely as great as the length of the more common unfertilized eggs. These eggs are more often than not triangular shaped as viewed under the microscope (figures 7, 8 and 9). That they are not always triangular is readily seen when they are rolled over. A bulge then appears on one surface but the egg can be placed so that it appears to be symmetrically formed around one major axis. About one to five per cent of the stools containing ascaris eggs were seen to have these triangular shaped eggs or eggs with bulges. In a number of cases these alone appeared on slides showing five or less eggs. Some of the eggs which appear symmetrical when seen in routine examination may also appear oddly shaped when viewed from a different

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\* Stoll dilution egg counting method wherein the eggs are counted in the small drop (0.075 cc.) of a 1 to 15 dilution of feces in N/10 sodium hydroxide (Stoll, 1923; Stoll and Hausheer, 1926).

angle. The finding of these eggs in N/10 sodium hydroxide solution suggested the possibility that this medium might be in some way exerting a distorting influence. However, Muira and Nishiuchi reported them from feces before the use of the Stoll method and from the uteri of adult worms and I have since found them under similar conditions.

To determine the relative importance of the unfertilized eggs diagnostically differential counts were made on ascaris eggs found in the Stoll small drop preparation from 820 stools. All types of unfertilized eggs were grouped together under one heading and considered in relation to the normal fertilized eggs. A total of 51,329 ascaris eggs were seen in these 820 preparations, an average of 63 eggs per slide of which 8155 or 15.9 per cent were unfertilized. Of these unfertilized eggs 2585 were found on 214 slides on which no fertilized eggs were

TABLE 1.—*Relative Distribution of Fertilized and Unfertilized Eggs in Stoll Small Drop Preparation of 820 Stools. The Preparations Are Grouped According to the Percentage of Fertilized Eggs Present and the Number and Percentage of Slides in Each Group Recorded*

Per Cent of Eggs Fertilized	Number of Slides in Each Group	Percentage of Slides in Each Group
0.....	214	26
1-10.....	16	2
11-20.....	14	1.5
21-30.....	14	1.5
31-40.....	9	1
41-50.....	22	3
51-60.....	25	3
61-70.....	17	2
71-80.....	37	5
81-90.....	66	8
91-99.....	126	15
100.....	260	32
Total.....	820	100

to be seen (Table 1), an average of 12 eggs per slide. However, most of the slides, whereon unfertilized eggs alone gave evidence of infestation, had less than 12 eggs per slide; 72 per cent having 10 or less and 51 per cent five or less. However, a number did have rather heavy infestations. There were nine cases in which 50 or more unfertilized eggs were the only evidence of heavy infestation. These counts were 53, 59, 62, 73, 76, 77, 128, 151, 202. There were still a larger number of high egg counts in which most of the eggs found were unfertilized. There were 13 cases having from 50 to over 200 eggs per, slide in which over 50 per cent of the eggs were unfertilized. Table 1 gives the distribution of cases according to the relative number of fertilized and unfertilized eggs present. The percentage having only unfertilized eggs, the two types mixed and only fertilized eggs (26, 42 and 32 per cent) is in general like that reported by Muira and Nishiuchi in 1902. They found that of the 35 cases seen 14 or 40 per cent had only unfertilized eggs; 4 or 14.3 per cent were mixed and 15 or 45.7 per cent had only fertilized eggs. From Table 1 it will be seen that



in only 48 per cent of the cases were 90 per cent or more of eggs fertilized while in 35 per cent of the cases all or more than half of the eggs were unfertilized. In the nine per cent of the cases where both types were present but more than half of the eggs were unfertilized the average egg count was 41, compared with the average count of 63 eggs per slide for the whole series of 820 cases.

The significance of the unfertilized eggs diagnostically has been demonstrated and it is interesting to consider their significance biologically. That these eggs are unfertilized eggs of *A. lumbricoides* and not degenerate eggs or eggs of another species is well established. Muira and Nishiuchi and others including the writer have removed single females or small numbers of females and no males from individuals whose stools contained only these eggs. The uteri of such females always contained these characteristic eggs. Muira and Nishiuchi and subsequent workers have studied the reproductive tract of females which had been associated with males. The lower part of the uteri usually contain either only the normal fertilized eggs or both fertilized and unfertilized eggs. In the vicinity of the seminal receptacle those eggs which later develop as the normal fertilized eggs are found surrounded and being penetrated by spermatozoa. Other eggs in which no spermatozoa can be demonstrated retain the characteristics of these unfertilized eggs as they pass down the uteri.

As already mentioned, solitary females, or several females without males, are found when a person passing only unfertilized eggs is treated. This may be due to the females never having been in copula or to the supply of spermatozoa received by copula having already been exhausted. It is difficult to determine the exact conditions of development but it seems probable in many cases that males have never been present with these isolated females. Perhaps in other cases males did develop but were dislodged without ever having functioned. Proof that copulation takes place more than once during the life of the worm is equally difficult to obtain. However, Looss (1905:113) and Herrick (1928:139-140) both suggest from experimental evidence with hookworm that it does take place more than once. Thus a female probably does not receive and store in the seminal receptacle enough spermatozoa from one copulation for the fertilization of all the eggs to be produced. Hence if a solitary male were to be dislodged after its first copulation the female might easily use up its supply of spermatozoa after a time and produce only unfertilized eggs thereafter. It is interesting to consider what has happened in those few cases mentioned where many unfertilized eggs alone, up to several hundred were found per slide, indicating about 20 or more females. No such cases were treated and hence one can only conjecture a shortage of males due probably both to shortage in the original infection and an early dislodgement of those which developed.



OTTO—UNFERTILIZED EGGS OF ASCARIS

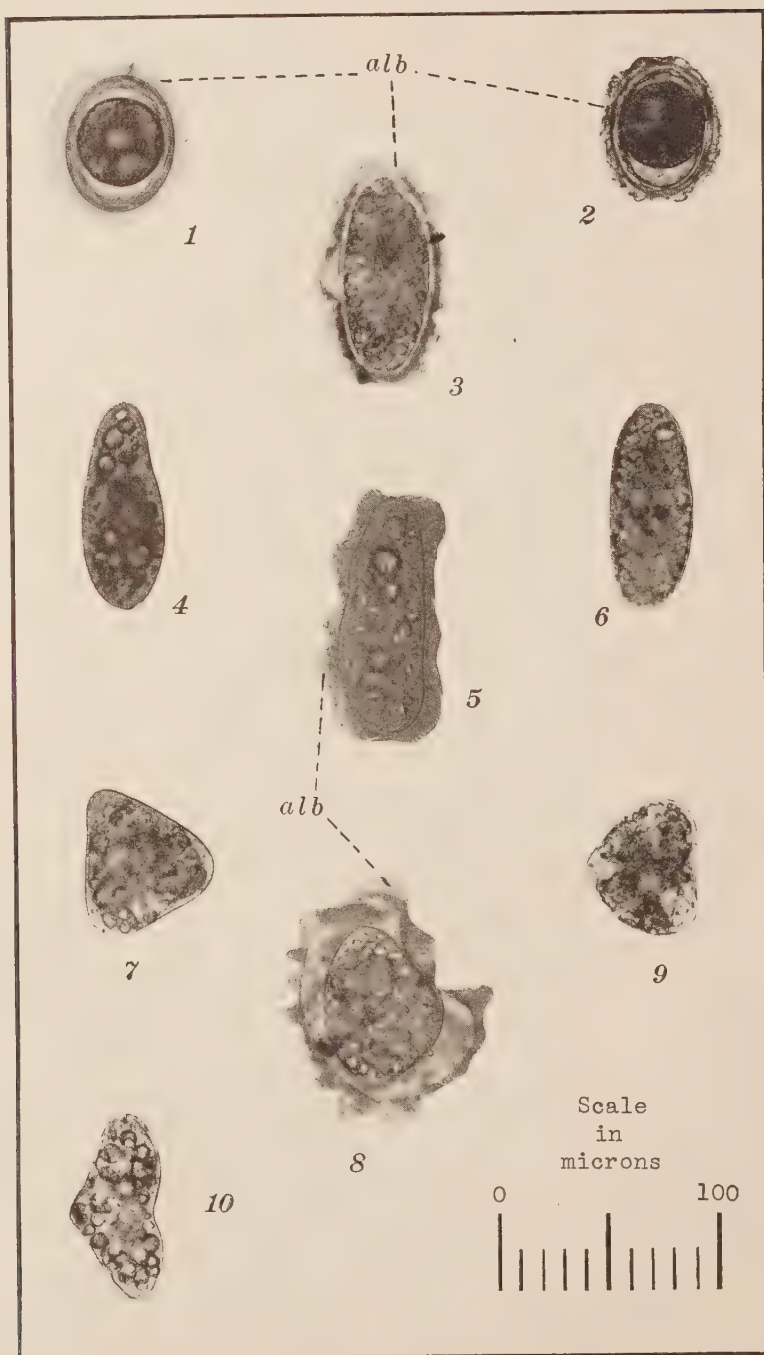


PLATE XXIV

When both fertilized and unfertilized eggs are found in the same infestation it is probably due in some cases to the presence of some females laying just unfertilized eggs and in other cases to the laying of both types of eggs by the same worm. The writer has found both types of eggs in the uteri on dissection and Muira and Nishiuchi used such worms for the basis of the histological studies on the nature of the unfertilized eggs. The proportion of worms laying both types of eggs and laying only one type must vary in the different individuals from day to day and any attempt to arrive at such a proportion from the egg count would be little more than a guess.

#### SUMMARY

Unfertilized eggs were found to represent 15.9 per cent of 51,329 ascaris eggs seen in 820 small drop preparations of the Stoll dilution egg count method. These eggs were found alone in 26 per cent of the slides, mixed in 42 per cent and absent in 32 per cent. They were distributed in high and low egg counts but most of the slides having just unfertilized eggs had low egg counts, the average being 12 and 72 per cent having less than 10 eggs, whereas the average egg count for the entire 820 slides was 63. Special attention is called to the asymmetrical unfertilized eggs and all are figured. Single female worms may be producing either only unfertilized or fertilized eggs at one time or both at the same time. Inasmuch as fertilization probably takes place more than once during a life time the production of worms producing one or both types of eggs must vary from time to time.

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#### EXPLANATION OF PLATE XXIV

Photomicrographs of eggs of *Ascaris lumbricoides* (Linn.)  $\times$  330.  
Abbreviation: alb., albuminous outer layer of shell.  
Figs. 1, 2.—Normal fertilized eggs.  
Figs. 3, 4, 5, 6.—Common types of unfertilized eggs.  
Figs. 7, 8, 9, 10.—Less common types of unfertilized eggs.

# OBSERVATIONS AND EXPERIMENTS ON THE OPALINID CILIATES OF THE GREEN FROG \*

ROBERT HEGNER

This is a continuation of studies begun a number of years ago (Hegner, 1922) because of the fact that the tadpole of green frogs and bull frogs have a high incidence of infection with opalinids, whereas the adults are usually free from these ciliates, although both tadpoles and adults of other American frogs are infected. The chief questions involved are (1) when and why do green frog tadpoles lose their opalinids and (2) why do not adult green frogs become reinfected? Green frogs, leopard frogs and tree frogs from Mount Desert Island, Maine, were studied during the summer of 1930 in an attempt to answer these questions.

Do adult green frogs on Mount Desert Island, Maine, harbor opalinids? This was the first problem undertaken. Ten adult green frogs of various sizes, and presumably of various ages, were examined. No opalinids were found. Ciliates of the genus *Nyctotherus* were present in the rectum of one specimen; trichomonads were present in large numbers in all; hexamitas were found in nine, but were few in number.

At what stage does the green frog lose its opalinids? To answer this question tadpoles were separated into three groups as follows: (1) without external legs, (2) with two posterior legs, and (3) with four legs. The lengths of body, tail and intestine were obtained for those with two and four legs and presented in the following table.

Number of Tadpoles	Number of Legs	Length of Body		Length of Tail		Length of Intestine	
		Range, Min.	Average, Min.	Range, Min.	Average, Min.	Range, Min.	Average, Min.
15	2	35-40	36	50-105	84	33-55	43
20	4	35-45	38	3-85	33	28-80	64

Every one of at least one hundred tadpoles without legs that were examined was infected with opalinids. All of the fifteen tadpoles with two posterior legs were likewise infected with opalinids. Large numbers were present in eleven of them and a few in the other four. *Nyctotherus* was noted in all but two and trichomonads and hexamitas in all. No opalinids nor *Nyctotherus* were found in the twenty tadpoles

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\* From the Mount Desert Island Biological Station at Salisbury Cove, Maine, and the Department of Protozoology of the Johns Hopkins School of Hygiene and Public Health.



with four legs. Eight of this group contained trichomonads and thirteen were infected with hexamitas.

These observations lead to the conclusion that green frog tadpoles lose their opalinids during metamorphosis between the two-legged and four-legged stages.

Do the tadpoles of other species of frogs retain their opalinids during metamorphosis or do they lose them at this time and become reinfected as young frogs? Data obtained in 1922 were inconclusive on this point. Three metamorphosing tadpoles of *Rana pipiens* and two of three young frogs of this species were found infected at that time. Five tadpoles of *Bufo americanus* with two visible legs were infected and five non-infected. In 1930, twenty-two tadpoles of *Rana pipiens* were examined for opalinids. Five tadpoles without legs were all well infected; eight with two legs were also well infected; and of nine with four legs, opalinids were found in three and absent from six. These results are also inconclusive. They indicate that the late stages in metamorphosis are unfavorable for the protozoan inhabitants of the rectum since *Nyctotherus*, trichomonads and hexamitas were also absent from three of this group. Probably opalinids are retained by some and lost by others, the latter becoming reinfected as adults.

The situation with respect to *Hyla versicolor* is more definite. Sixteen tadpoles of this species were examined, three with two small legs, three with two large legs, and ten with four legs. Every specimen contained a good infection with opalinids indicating that these ciliates remain present throughout the period of metamorphosis of this species.

Can recently metamorphosed green frogs be reinfected with opalinids from green frog tadpoles? Forty-seven frogs were used in an attempt to answer this question. Material containing opalinids taken from the rectum of green frog tadpoles was injected at once into the stomach of eight of these frogs. Opalinids and the other intestinal protozoa present in the inoculum were found in the stomach of one frog one and one-half hours after injection in apparently a normal condition. None had reached the rectum. Five frogs were killed and examined four hours after the injection. Opalinids were still present and viable in the stomach of two of these but none were found in the rectum. Two frogs were kept twenty-two hours before examination; these were both negative throughout for opalinids. It seems evident that trophozoites of opalinids from green frog tadpoles are unable to pass through the stomach and intestine and set up an infection in the rectum of young green frogs. This might have been possible if cysts had been used instead of trophozoites.

In thirty-nine of the young green frogs opalinids from green frog tadpoles were inoculated into the rectum and the frogs killed at intervals

of from 2 to 96 hours. Two of the frogs died; the data for the other thirty-seven are as follows:

Number of Frogs	Interval Between Inoculation and Examination, Hours	Opalinids
1	2	Negative
2	2.5	Two positive; both with a few
4	5	Two positive; one with one, other with many
4	21	Two positive; one with two, other with few
4	24	One positive, with several
8	48	Two positive; one with two, other with several; all alive but not swimming about
5	50	One positive, with few quiescent
4	72	One positive; with two quiescent
5	96	Negative

Although these opalinids were inoculated in large numbers directly into the rectum, which is their optimum habitat in other species of frogs, only eleven of thirty-seven retained them and they were few in number compared with their abundance in the inoculum. In four frogs the opalinids persisted in very small numbers for from forty-eight to seventy-two hours, but were not swimming about, although they were alive, as indicated by the movement of their cilia. Some of them were evidently abnormal. Under these conditions it seems impossible to colonize the rectum of recently metamorphosed green frogs with opalinids living normally in green frog tadpoles. It might be possible to induce other species of opalinids to live in the rectum of green frogs, but no attempt was made to do so.

Why do green frog tadpoles lose their opalinids at the time of metamorphosis and metamorphosed green frogs resist infection? In a previous report (Hegner, 1922) experiments on green frog tadpoles with different diets led to inconclusive results, although a largely meat diet and a diet of thyroid substance seemed detrimental to the ciliates. Whether the decrease in the number of opalinids under these conditions was due to the direct action of the diet or to the speeding up of metamorphosis could not be determined. It is suggested that diet is not the controlling factor but that digestive secretions produced by the green frog render the rectum an unfavorable habitat for the opalinids.

#### CONCLUSIONS

Tadpoles of the green frog on Mount Desert Island, Maine, are heavily infected with opalinids, whereas newly metamorphosed frogs and older frogs are not. The tadpoles lose their opalinids during metamorphosis between the two-legged and four-legged stages. Some tadpoles of *Rana pipiens* seem to retain their opalinids during metamorphosis; others apparently lose theirs and become reinfected after metamor-

phosis. Tadpoles of *Hyla versicolor* appear to retain their opalinids throughout metamorphosis. The trophozoites of opalinids from the rectum of green frog tadpoles are unable to pass through the stomach and intestine of metamorphosed green frogs and reach the rectum in a viable condition. Cysts might be able to do so. Opalinids from green frog tadpoles injected directly into the rectum of metamorphosed green frogs are unable to set up infections. It is suggested that the intestinal secretions of the green frog render the rectum of this species unfavorable for opalinids.

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## ON THE PRESENCE OF PERIPHERAL CHROMATIN IN *ENDOLIMAX NANA*

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*Limax*-amoebae have been described from a large series of hosts, for example: oysters; cockroaches; termites; larvae of *Phyllophaga* sp., crane-flies and Harlequin flies; rock-fish; frogs; lizards; domestic fowl; domestic turkey; rats; man and others. In the descriptions of these various amoebae is found more or less general agreement regarding certain features of the nuclear structure. The karyosome is uniformly recorded as being of large size and staining intensely with hematoxylin. In some of the species spoke-radii are reported to connect the karyosome with the nuclear membrane. The greatest discrepancies, however, occur in the observations on the peripheral chromatin, some forms being recorded as having an abundance, while none was noted in others.

In the form from man, *Endolimax nana*, the nucleus is described as having a large, darkly staining karyosome, with spoke-radii extending to the nuclear membrane, on which is to be found no true peripheral chromatin. In view of the differences of opinion regarding the nuclear structure of the *limax*-amoebae, some observations on the nucleus of *Endolimax nana* seemed worthy of presentation. The writer is grateful to Dr. D. H. Wenrich of the Department of Zoology of the University of Pennsylvania, for guidance and criticism.

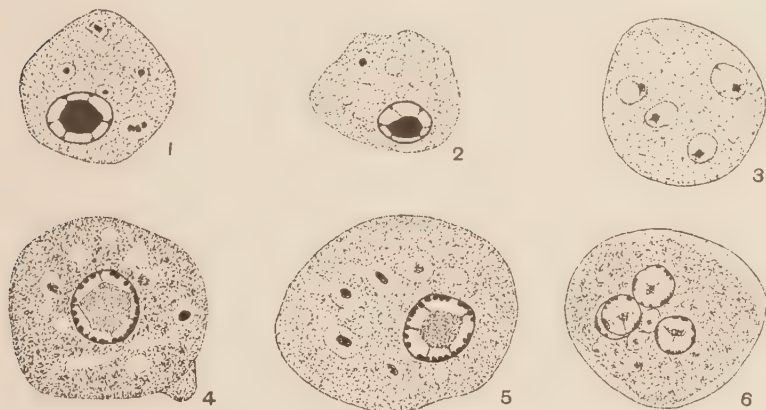
The material for these observations was obtained from numerous human cases infested with the trophozoites and cysts of *Endolimax nana*. It was prepared in a variety of ways, including fixation in Schaudinn's fluid plus 5 per cent and 20 per cent glacial acetic acid. The staining was done entirely with Heidenhain's hematoxylin.

### OBSERVATIONS

Study of the trophozoites of *Endolimax nana* reveals that when they are fixed in Schaudinn's plus 5 per cent glacial acetic acid, the following nuclear picture is produced. The karyosome is large, homogeneous and stains very intensely with hematoxylin, being one of the last elements to lose its stain on differentiation. Spoke-radii connect it with a faint nuclear membrane, on which the only apparent chromatin is arranged as small lumps at the distal ends of these radii (Text fig., 1 and 2). However, when 20 per cent glacial acetic acid is added to the fixative, a marked difference from the above is noticed. The karyosome

now stains lightly and rapidly loses color with even moderate exposure to differentiation. The radii are present, but the nuclear membrane is now readily seen to be encrusted with a uniform ring of small granules (4 and 5).

In the cysts, the same results are obtained, but, due to the general reduction in amount of nuclear chromatin on encystment in *Endolimax nana*, the effects of the 20 per cent acetic are more difficult to observe. In Schaudinn's plus 5 per cent acetic, the karyosomes are dark, but the radii and distal chromatin lumps are usually not prominent (3), while in Schaudinn's plus 20 per cent acetic the karyosomes are again faint and careful observation reveals a series of chromatin lumps on the periphery of the nucleus (6).



Text Figure.—Camera lucida drawings of *Endolimax nana*. Description in text.  $\times 3,000$ .

These observations were confirmed many times. Smears from the same small bit of feces, treated identically save for the difference in acetic acid content of the fixative, yielded the results pictured in the text figure, where numbers 1, 2, 4 and 5 are from slides made from the same small fecal mass. The cysts, numbers 3 and 6, are from another case, similarly treated.

#### DISCUSSION

The early descriptions of what is now known as *Endolimax nana*, by Gauducheau (1908), Elmassian (1909), Wenyon (1912, 1913, 1915 and 1916), Chatton and Lalung-Bonnaire (1912), James (1914) and others, agree in that the organism was not regarded as a true parasite of man. The nucleus was described as having a large karyosome, a very faint nuclear membrane, on which there was little or no peripheral chromatin.



Since the true status of the amoeba has been recognized, the works of Swellengrebel and Winoto (1917), Wenyon and O'Connor (1917), Dobell and Jepps (1917), Kuenen and Swellengrebel (1917), Brug (1918), Dobell (1919) and Wenyon (1926) have added little to the early conception of the nuclear structure, except for the observation of the spoke-radii and the small lumps of chromatin at the distal ends of these radii. Dobell (1919) states that the nucleus has "a well-marked nuclear membrane in which minute granules—possibly of chromatin—can sometimes be seen" and that he has not been able to convince himself "of the existence of any 'peripheral chromatin' in the clear zone" between the nuclear membrane and the karyosome. Wenyon (1926) asserts that there "is a definite nuclear membrane which appears to be free from chromatin, all of which seems to be concentrated in the karyosome."

The present study shows the existence of an abundance of this so-called peripheral chromatin in the trophozoites of *Endolimax nana*, the proper technique only being necessary to demonstrate it clearly. This is in agreement with some of the results on the *limax*-amoebae from other hosts. Mackinnon (1914) described peripheral chromatin in *Vahlkampfia* sp. from the Crane-fly larva. Lucas (1927) saw peripheral chromatin in one specimen of *Endolimax blattae* from the cockroach. In *Endolimax termitis* from *Mirotermes hispaniolae* Kirby (1927) records an abundance of this chromatin. Hogue (1915), though failing to find chromatin on the nuclear membrane in *Vahlkampfia calkensi* from oysters, did note it (Hogue 1921) in *Vahlkampfia patuxent*, also from the oyster. Others, Dobell (1914) in *Amoeba lacertae* from *Lacerta muralis*, Tyzzer (1920) in *Pygolimax gregariniformis* from the domestic fowl and turkey, Chiang (1925) in *Endolimax ratti* from laboratory rats, make no mention of this chromatin, or state definitely that none is present.

These references do not represent a complete list, but suffice to show the varied observations on the nuclear structure of the *limax*-amoebae. The suggestion presents itself that, as in the case of *Endolimax nana* where no true peripheral chromatin was stated to be present, a variety of techniques would be most desirable in further studies on these amoebae and they may be found to be more similar morphologically than previously described.

In conclusion, the results herein recorded show a closer relationship between *Endolimax nana* and the species of Entamoeba, in which peripheral chromatin is characteristic. Definite physical or chemical differences are suggested by the differential behavior of the karyosome and peripheral chromatin of *Endolimax nana* to the change in the acetic acid content of the fixative. These two elements do not react

alike and therefore must be different. The nature of this difference is still to be determined.

#### SUMMARY

1. The typical picture of *E. nana* is produced by fixing in Schaudinn's fluid plus 5 per cent glacial acetic acid; Schaudinn's fluid plus 20 per cent glacial acetic acid produces notable differences: The karyosome retains hematoxylin poorly; A definite ring of peripheral chromatin appears on the nuclear membrane.

2. A difference in composition is indicated between the karyosomal and peripheral chromatin.

3. The presence of the peripheral granules makes Endolimax a close relative of Entamoeba.

4. The use of a variety of techniques is suggested for the study of these amoebae.

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A COMPARATIVE STUDY OF THE MALE  
TERMINALIA OF CALIFORNIAN  
ANOPHELINES

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The terminal segments of the mosquitoes were clipped from anesthetized specimens and placed in a watchglass containing a bit of cotton soaked in 30 per cent alcohol. They were immediately mounted on slides in Gater's medium and were allowed to clear for forty-eight hours or more before they were examined. Camera lucida drawings were then made of the terminalia which are diagrammatic to the extent that they are bilaterally symmetrical, whereas, due to difficulties in mounting, only a few of the mounted specimens show this symmetry. The processes of the ninth tergite and the anal lobe have been omitted from the drawings as they are apt to obscure the other structures of greater taxonomic importance.

*Anopheles pseudopunctipennis* THEOBALD [TEXT FIGURE A]

Side-piece, almost twice as long as width at base.

Internal spine, prominent, about half-way distad of the middle of the side-piece.

Parabasal spines, two, the inner spine not as long as the outer spine and recurved at tip, both spines taper to a point.

Clasper, longer than side-piece and slightly constricted in the middle, apical claw short.

Claspettes, bilobed, the ventral lobe dome-shaped, with two stout tapering setae at apex; the dorsal lobe elongate with three strong setae.

Mesosome, cylindrical with a slight bulge at apex, apical opening without leaflets. No specimen of our species of *pseudopunctipennis* yet examined has had the "four, delicate, serrate 'leaflets'" noted by Root (1924) on specimens from Mexico.

*A. maculipennis* MEIGEN [TEXT FIGURE B]

Side-piece, at least one and one-half times as long as wide.

Internal spine, slightly distad of middle.

Parabasal spines, two, stout; outer spine long, tapering to a point; inner spine broad, recurved at tip.

Claspers, longer than side-piece, constricted medianly.

Claspettes, bilobed, the dorsal lobe small with two pointed spines, the ventral lobe larger with two or three spines, usually the latter.



A



B



C

Mesosome, stout, cylindrical, with three pairs of apical leaflets, the pairs of leaflets becoming progressively longer posteriorly.

*A. punctipennis* SAY [TEXT FIGURE C]

Side-piece, nearly twice as long as wide, conical.

Internal spine, prominent, arising one-third from the apex of the side-piece.

Parabasal spines, two, short, stout, arising from a prominent tubercle; the inner spine recurved at tip.

Clasper, longer than side-piece, apical claw short, stout.

Claspettes, bilobed, dorsal lobe small, with two closely set, short, stout spines; ventral lobe much broader with an outer large, broad and sharp-pointed spine, an inner smaller spine and midway between these spines a hair.

Mesosome, more slender than that of *maculipennis* but longer and stouter than that of *pseudopunctipennis*; apically three pairs of approximately equal leaflets, more slender than those of *maculipennis*.

#### CONCLUSIONS

The terminalia of our Californian Anophelines may be used for taxonomic purposes. The salient points of differentiation are: the length of the side-pieces, the point of origin of the internal spine, the leaflets of the mesosome and, most important of all, the morphology of the claspettes

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TRICHOMONAS PHASIANI, A NEW FLAGELLATE FROM  
THE RING-NECKED PHEASANT, PHASIANUS  
TORQUATUS GMELIN\*

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An examination of fecal material from the cecum of a female pheasant revealed the presence of myriads of trichomonad flagellates. Intestinal smears were fixed in Schaudinn's fluid and were stained in Heidenhain's iron-hematoxylin. In 1929 Tyzzer published a paper on the Coccidia of gallinaceous birds and described *Eimeria phasiani*, but there seems to be no record of a flagellate from this host. Donné in 1937 established the genus *Trichomonas* with *T. vaginalis* as the genotype. He did not accurately determine the number of flagella, but Künstler in 1884 and Lynch in 1915 state that there are four anterior flagella in this species. Kofoed (1920) retains *Trichomonas* for those trichomonads possessing four anterior flagella.

The trichomonads described from birds are: *Trichomonas columbae* Rivolta (1878) from the pigeon, *T. gallinarum* Martin and Robertson 1911 from the chicken, *T. anatis* Kotlan 1923 from the duck, *T. oti* Tanabe 1926 from the screech owl, *T. avium* Cunha and Muniz 1926 and *T. lanceolata* Cunha and Muniz 1926 from Brazilian birds, *T. flordinae* Hegner 1929 and *T. ortyxis* Hegner 1929 from the valley quail, *T. anseri* Hegner 1929 from the goose, *T. diversa* Volkmar 1930 from the turkey, *Tritrichomonas eberthi* Martin and Robertson 1910 from the chicken, and *T. bonasae* Tanabe 1926 from the grouse.

*Trichomonas phasiani* N. SP.

This flagellate is slender pyriform with a body curvature varying according to the arcuation of the chromatic basal rod (Fig. 1). The anterior tip of the organism is obtusely rounded. The posterior end tapers to a point. The cytoplasm stains lightly with hematoxylin. A large cytostome is present on the ventral side near the origin of the flagella.

A single large blepharoplast is situated at the extreme anterior margin of the body. Martin and Robertson (1911) were able to distinguish four basal granules in *T. eberthi*, but only one can be demon-

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\* The writer is deeply indebted to Dr. E. R. Becker for his encouragement and help in the preparation of this paper.

strated in this species. Five flagella and a chromatic basal rod arise from the blepharoplast. Four of the flagella are anterior and subequal in length; two as long as, or slightly longer than the body; one of medium length slightly shorter than the long ones; one distinctly shorter than the one of medium length (Fig. 3). The fifth flagellum runs along the outer margin of a well developed undulating membrane and extends free caudally about half the length of the body. The chromatic basal rod is filiform, stains darkly with hematoxylin, and curves dorsally from the blepharoplast to the posterior end of the body, ending near the axostyle. The axostyle is slender, hyaline, and quite inconspicuous, projecting slightly and ending in a point. It stains so lightly with hematoxylin that it can seldom be traced to its origin, the blepharoplast.

The ellipsoidal nucleus located just behind the basal granule is almost indistinguishable, staining very lightly with hematoxylin. It is surrounded by a thin membrane and seems to be filled with minute chromatin granules. The karyosome is small and may be either centrally (Fig. 1) or eccentrically (Fig. 2) placed with a definite clear area or achromatic capsule surrounding it. A few specimens are present with nuclei that stain an even gray, which may be caused by diffuse chromatin (Fig. 5). No cysts were observed.

Measurements of 500 individuals on stained slides are presented in the correlation table.

TABLE 1.—Length and Width Correlation of Trophozoites of *Trichomonas phasiani*, n. sp.

Width in Microns	Length in Microns													Total
	5.5	6	6.5	7	7.5	8	8.5	9	9.5	10	10.5	11	11.5	
1.5.....	1	..	..	1	..	..	1	..	..	..	..	..	..	3
2.0.....	1	..	2	3	14	3	8	2	..	..	..	..	..	33
2.5.....	..	..	3	6	17	9	24	12	4	3	1	..	..	79
3.0.....	..	..	..	18	37	26	69	50	12	22	8	..	1	243
3.5.....	..	..	..	4	12	6	24	20	8	13	2	..	..	89
4.0.....	1	..	..	..	5	1	6	17	3	8	3	..	..	44
4.5.....	..	..	..	..	..	1	..	3	2	1	1	..	..	8
5.0.....	..	..	..	..	..	..	1	..	..	..	..	..	..	...
Total.....	3	..	5	32	85	46	133	104	29	47	15	..	1	500
									Length		Width			
Range .....									5.5-11.5 $\mu$		1.5-5 $\mu$			
Mean .....									8.519 $\mu$		3.051 $\mu$			
Standard deviation .....									0.943 $\mu$		0.541 $\mu$			

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- Other references cited will be found in Wenyon.

EXPLANATION OF PLATE XXV

× 4,000

- Fig. 1.—Typical trophozoite.
- Fig. 2.—Dorsal view of a trophozoite.
- Fig. 3.—Trophozoite illustrating flagellar lengths.
- Fig. 4.—Trophozoite with long axostyle.
- Fig. 5.—Trophozoite with a dark staining nucleus.
- Fig. 6.—Trophozoite with distorted axostyle and two blepharoplasts.



PHYSALOPTERA POLYDENTATA N. SP.\*

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The material upon which this species is based consisted of male and female specimens taken from a Gecko (*Hemidactylus mabouia*) obtained in Tanganyika Territory, British East Africa, and placed at my disposal through the courtesy of Dr. H. A. Baylis of the British Museum (Natural History).

The cuticula shows a very delicate transverse striation and is partially reflexed over the lips of both sexes. The excretory pore opens about 10 to 20 $\mu$  back of the cervical papillae which are located opposite the cephalic end of the posterior division of the esophagus. The two lateral lips are large and rounded in lateral view and are sharply set off from the body. Each lip carries a pair of large sub-median papillae set deeply in the greatly inflated cuticula. Each lip is provided with a large outer and a small inner median tooth and a pair of inner teeth at each lateral angle. These lateral teeth are but slightly differentiated in size and position from the very distinct dentigerous ridge of from five to six members which extends almost the entire distance from the inner tooth to the lateral pair.

The esophagus is straight, opens directly into the mouth, shows definitely an anterior glandular and a posterior muscular portion, and measures 1/7 of the total length of the female and 1/6 of that of the male. The nerve ring surrounds the posterior fifth of the anterior portion of the esophagus.

The number of uteri is very definitely four, which places this form in the group Tetradelphys as established by Ortlepp (1922). The vulva opens at about the level of the posterior end of the esophagus, usually slightly caudad of that position. From the vulva to the division of the common trunk into the two primary branches is about 1/9 of the body length i. e., 3.5 mm, and from the distal end of the common trunk to the secondary branches about 0.5 mm. The secondary branches of the uteri are extremely intertwined and difficult to follow to their respective ovaries.

The male bursa is long and pointed, with a slight ventral flexion. The under surface is ornamented by a cluster of pre-cloacal superficial disc-like markings which closely approximate in their position the pre-cloacal sessile papillae. The four pairs of stalked papillae are evenly spaced, the two middle pairs being slightly the longer. Two pairs are

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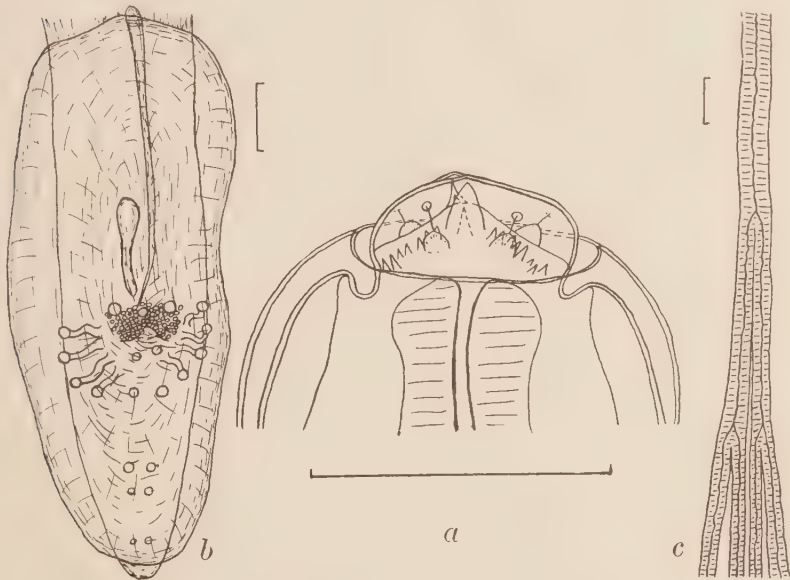
\* Contribution from the Biological Laboratories of Knox College, No. 42.

pre-cloacal, and two pairs post-cloacal in position. The three pre-cloacal sessile papillae are of about equal size, the middle one being slightly nearer the cloacal lip. The post-cloacal papillae are in three groups; pairs 1 & 2, 3 & 4, and pair number 5. The first and last pairs are somewhat smaller than the others.

The spicules are very unequal, the left being long and slender while the right one is short and stout.

The detailed measurements are as follows:

*Male:* Body length, 12.65 mm.; greatest width, 0.4 mm.; length of anterior esophagus, 0.375 mm.; length of posterior esophagus, 1.9 mm.;



DESCRIPTION OF TEXT FIGURE

*Physaloptera polydentata* n. sp. *a*, anterior end of male; *b*, tail of male, ventral aspect; *c*, diagrammatic sketch of the division region of the ovarian ducts. Scale in all figures equals 0.1 mm.

head-cervical papillae distance, 0.39 mm.; head-excretory pore distance, 0.4 mm.; head-nerve ring distance, 0.25 mm.; cloaca-tail distance, 0.4 mm.; length of spicules, left—0.425 mm., and right—0.1625 mm.

*Female:* Body length, 30 mm.; width at vulva, 0.45 mm.; length of anterior esophagus, 0.425 mm.; length of posterior esophagus, 4 mm.; head-cervical papillae distance, 0.51 mm.; head-excretory pore distance, 0.52 mm.; head-nerve ring distance, 0.39 mm.; head-vulva distance, 3.5 mm.; anus-tail distance, 0.5 mm.; size of ova, 0.025 mm. by 0.05 mm.

*Habitat:* Intestine.

*Host:* *Hemidactylus mabouia* (Gecko).

*Range:* Tanganyika Territory, British East Africa.

*Type Specimens:* No. 10.23.138-142.1929. Helminthological Collections of the British Museum (Natural History).

This form, possessing as it does, the tetradelphys form of uteri and also dentigerous ridges, is closely related to *Physaloptera quadrovaria* Leiper (1908), *P. paradoxa* v. Linstow (1908), and *P. pallaryi* Seurat (1917). It is easily distinguished from these three species by the differences in the relative proportions of the various divisions of the uterine structures, by the plan and ornamentation of the male bursa, and by the number and arrangement of the denticles. This form resembles most closely *P. quadrovaria*, but even in this case the separation is easily made on the plan of subdivision of the common trunk of the oviduct; the primary divisions in *quadrovaria* being almost entirely eliminated while those of *polydentata* are of considerable length.

This form has been called *Physaloptera polydentata* n. sp. because of the presence of dentigerous ridges, a condition not of common occurrence in the tetradelphoid forms of this genus.

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# A NEW LARVAL CESTODE, PROBABLY *HYMENOLEPIS CUNEATA*, A TAPEWORM OF A WILD DUCK

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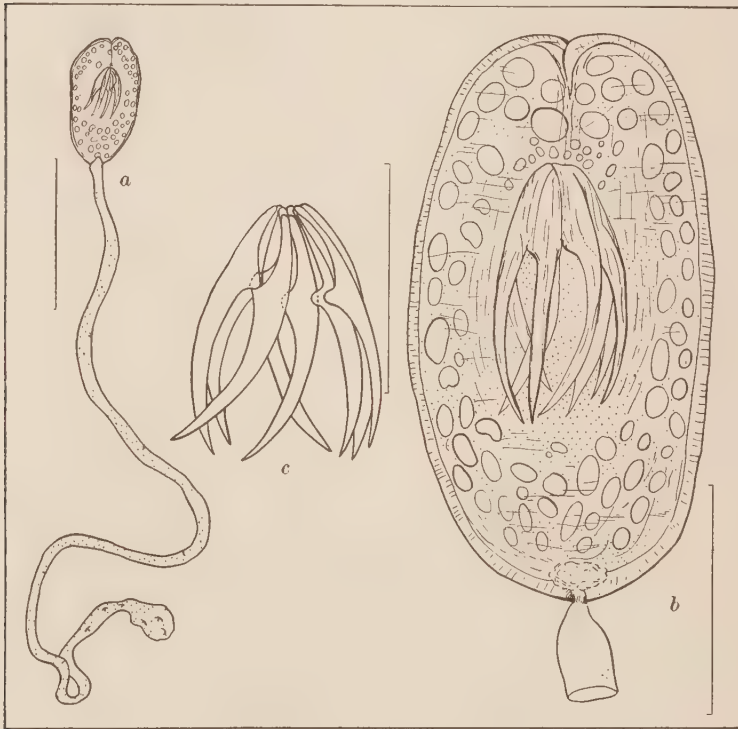
During the summer of 1927 while engaged in a field study on the life history and frequency of *Diphyllbothrium latum*, organized by Dr. Henry B. Ward and supported by a grant to him from the Committee on Medical Research of the American Medical Association (Ward, 1927, 1929), and in connection with another investigation I was making (Essex, 1927), a large number of copepods from Long Lake, Ely, Minnesota, were examined for larval tapeworms. Magath first called my attention to an interesting proceroid in the body cavity of *Diaptomus oregonensis*. This parasite was subsequently seen in two other copepods of this species.

When liberated from the body cavity of the host the parasite was markedly quiescent as compared to the great activity exhibited by the larvae of certain other species which I have observed. An examination of the larvae revealed that they were composed of two distinct body regions, the body proper and an exceedingly long caudal appendage or cercomer. The body portion which measured 0.23 by 0.13 mm., was ovate, and the anterior margin in the terminal region turned in toward the middle where a set of hooks could be distinguished (Figs. *a* and *b*). In the living specimen only four hooks could be seen distinctly, but after the organism had been allowed to disintegrate slightly eight hooks could be clearly seen (Fig. *c*). The hooks, which measured about  $110\mu$  in length and about  $22\mu$  in their broadest portion, were arranged roughly in four pairs. Acetabula were not observed. Scattered throughout the body portion were many refractile bodies of varying size and shape. Such bodies were not seen in the cercomer. This part of the organism measured about 1.8 mm. in length with an average diameter of about 0.03 mm. The terminal portion was somewhat broadened and bore the six cast-off hooks of the oncosphere.

## DISCUSSION

The hooks of parasitic worms are considered one of the most reliable diagnostic characters in the identification of species. When such structures are present in a species it is possible to identify the larval form with the adult parasite with a reasonable degree of accuracy. The character of the hooks of the larva just described indicates that the adult

form is not a tapeworm of fish. An inspection of the hooks immediately suggests the genus *Hymenolepis*. Since certain of the species belonging to this genus are harbored by aquatic birds a comparison of the hooks with those of the known avian species is the logical procedure. It is fortunate that an extensive study of this group has been published by Mayhew (1925). Due to his detailed record of the salient morphologic features of previously described and new American species it has not



TEXT FIGURE

Camera lucida tracings. *a*, proceroid immediately after removal from body cavity of *Diaptomus oregonensis*; *b*, body portion of proceroid much enlarged; *c*, hooks after organism had become slightly disintegrated. Scale in *a* equals 0.3 mm.; in *b* and *c*, 0.1 mm.

been a difficult task to refer the larva in question to the probably adult form. A study of the text and figures on the different species of *Hymenolepis* described by Mayhew revealed that only one species possessed hooks that answered the description of those borne by this larval cestode. On the basis of the close similarity of the hooks of the proceroid under consideration to those of *Hymenolepis cuneata*,



(Mayhew, 1925) it is reasonably safe to identify this larval tapeworm as the proceroid of a species of *Hymenolepis* probably *H. cuneata*, a tapeworm of a wild duck.

This report is being made in the hope that it may be of service to some future worker who may be interested in the life cycles of the tapeworms of aquatic birds.

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## PORCUPINE LOUSE INFESTING THE MONKEY\*

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A *Macaque rhesus* monkey suffering from a severe dermatitis was brought to the Veterinary Department of the University of Minnesota from the Como Zoological Gardens of St. Paul on May 9, 1931. Upon examination an unusually heavy infestation of biting lice (Mallophaga) was found but no sucking lice, which are quite common on monkeys, were observed.

A collection of lice made from this animal consisting of 7 males, 7 females and 4 immature specimens after clearing the material has been identified as *Eutrichophilus setosus* (Giebel) Mjoberg, the porcupine louse. This is a common parasite on the Canada porcupine, *Erethizon dorsatum*, and has been reported from Lake and St. Louis Counties of Minnesota by Jellison (1931). A cage of porcupines was maintained in the gardens near the monkeys and is the probable source of this infestation. Six species of Mallophaga that have been found on various primates are recorded by Stiles (1929) but these were believed to be on their normal hosts. The degree of infestation, especially of immature specimens, found in this instance indicates that the porcupine louse was well established on its new host.

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\* Paper No. 1083 Scientific Journal Series of the Minnesota Agricultural Experimental Station.

A NEW PATHOGENIC NEMATODE OF THE FAMILY  
OXYUROIDEA, *OXYURONEMA ATELOPHORA*  
n. g. n. sp. IN THE RED-SPIDER MONKEY,  
*ATELES GEOFFROYI* \*

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Rockefeller Foundation Fellow

A red-spider monkey, *Ateles geoffroyi*, from Panama, was positive for *Strongyloides* spec. daily for a period of 3½ months. This infection was rather heavy. On August 31, 1931, *Balantidium coli* was found in the stool; subsequently it was found every two or three days. On July 11, 1931, an oxyurid-like nematode appeared in the feces for the first time and was found again September 8. On September 14 the feces of the monkey contained fresh blood, which indicated intestinal hemorrhage. The monkey became gradually weaker from this time on; it could not stand up on the morning of September 16, 1931, was in agony at ten o'clock that night, and died the next morning at 8 o'clock.

Autopsy revealed that in addition to the filariid worm, *Acanthocheilonema* spec., in the peritoneal cavity and *Strongyloides* spec. in the intestinal wall, from the pylorus to the end of the ileum, the cecum and rectum were filled with hundreds of specimens of an oxyurid-like species, mostly attached to the mucosa. Examination of these parasites showed that they belong to a new genus, which is readily differentiated from the known oxyurid species, and that the parasite was in part if not largely responsible for the death of the host.

OXYURONEMA nov. gen.

Body plump, gradually tapering at both ends. Female much longer than male. Posterior end of female elongated; of male, truncated rounded. Cuticula with several layers, annulated. Three lips. Mouth cavity present, with cuticular skeleton. Amphids present, openly elliptical in shape. Esophagus with two bulbs and three movable spears, which are connected with well-developed muscular apparatus in end bulb. Female with only one ovary, which is reflected anteriorly. Vulva in first third of body. Eggs with double shell, very numerous. One reflected testis. One spiculum. Gubernaculum not observed. Anal armament of male consisting of four pairs of papillae, which can be contracted by muscles in cuticula, and terminal spine, which has secretory canal. *Habitat*: parasitic in rectum of *Ateles geoffroyi*.

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\* Contribution from the Parasitology Laboratory, Department of Tropical Medicine, Tulane University, under the direction of Ernest Carroll Faust.

## OXYURONEMA ATELOPHORA n. g., n. sp.

*Size:*1. *Mature female*:  $n = 10$ 

$$L = 4.673 - 5.455 (5.156)$$

$$\alpha = 12.8 - 15.0 (14.2)$$

$$\beta = 5.5 - 6.5 (6.1)$$

$$\gamma = 4.1 - 5.5 (4.8)$$

2. *Old mature female*:  $n = 2$ 

$$L = 7.375 - 8.880 (8.127)$$

$$\alpha = 12.1 - 12.7 (12.4)$$

$$\beta = 15.2 - 15.8 (15.5)$$

$$\gamma = 4.3 - 4.9 (4.6)$$

The body of *Oxyuronema atelophora* is rather coarse and tapers gradually at both ends. The anterior part is  $\frac{1}{3}$  to  $\frac{1}{5}$  of the body width at the posterior end of the esophagus or  $\frac{1}{5}$  to  $\frac{1}{7}$  of the greatest body width. The head is distinctly set off from the trunk. In the younger mature female it is contracted (Fig. 1); in the old mature female (Fig. 2) it is swollen. The posterior end is elongated. The cuticula shows a very distinct annulation, which begins in front of the mouth cavity and gradually disappears towards the posterior end of the body. Thickness:  $3.75$  to  $15\mu$  ( $8.2\mu$ ). It is composed of different layers. At the anterior end (Fig. 1 a) there are seven layers; the third layer from the outside is enlarged, as is also the sixth; the inner layer is thickest. Behind the head posterior to the widening of the body (Fig. 1 b) the number of the layers decreases to five, in one case to six; the innermost layer is the widest. In both cases there is a distinct separation between the two inner layers. The single annules are distinctly developed in the anterior part of the body. Towards the middle of the body the annulation becomes less distinct (Fig. 1 c). Here the number of layers is six; layer four is the widest. Towards the posterior end of the body the annulation disappears (Fig. 8 d), being completely absent in the tail. The number of layers finally decreases to three. The annules themselves can be seen distinctly only in the anterior part of the body. Towards the first bulb they vanish gradually until they can no longer be observed posterior to the esophagus. This is a very striking difference in comparison with *Enterobius vermicularis*, where the annulation is visible along the whole length of the body. Another difference is that the annules in *Oxyuronema* appear as continuous lines, whereas in *Enterobius vermicularis* they are "dotted" lines.

The musculature (*mu*) is very well developed and consists of the following parts: Body musculature. This consists of numerous longitudinal fibers which are arranged in the form of a cylinder. The muscles begin just behind the head and extend to the end of the tail. These produce contraction of the body. Anal musculature (Figs. 8, 9;

*anmu*). This is arranged radially in front of the anus. Musculature of the lips (Figs. 3, 4, *linu*). It is at the anterior base of the lips and consists of ring muscles, which serve to open and close the whole apparatus of the lips. The muscles are very well developed, not only in the female but also in the male. In end view they are arranged in circular fashion around the mouth opening. It is possible that this musculature is used in the fixation of the nematode in the intestine, so as to press the lips against the wall of the intestine. Under these circumstances the lips have the function of a suctorial organ. Musculature of the posterior bulb of the esophagus (Figs. 1, 2, 7, *bumu*). This system is very strongly developed. Its function will be described later.

The head (Figs. 2-5). It has already been mentioned that the form of the head depends upon the age of the nematode. Although it is spherical in the old mature female (Fig. 3), in the younger mature female the head is narrower anterior to the neck. On account of the enormous elongation of the ovary in the old female the esophagus and the other anterior organs probably undergo a shortening. It is conceivable that the head does not grow any longer; consequently it expands laterally under the pressure of the growing sex organs. The mouth cavity (*mc*) is small, a little wider than long; size: 15 to 20 $\mu$ :17.5 to 25 $\mu$  (19:20 $\mu$ ) in the younger female and 10 to 15:17.5 to 20 $\mu$  (12.5:19 $\mu$ ) in the old female. The influence of the shortening of the anterior part of the body is also seen here. The proportion of the length to the width in the older female is approximately 2:3, while in the younger form it is very close to 1:1. In the inner part of the mouth cavity there is a cuticular skeleton (*mcsk*) which seems to be in connection with the spears. Teeth are absent. The skeleton consists of irregularly formed rod-like pieces, which apparently are movable. They are also connected with the lips and are probably reinforcements of the whole suctorial apparatus. The amphids (Figs. 3, 5 *am*) are very small and very difficult to see; size: 3 to 4 $\mu$  or  $\frac{1}{19}$  to  $\frac{1}{20}$  of the corresponding width. At first glance they appear elliptical, but careful examination shows that they are not closed. The amphids are just in front of the labial muscles.

The esophagus (Figs. 1, 2, 6 *oe*) consists of two parts, the first is cylindrical and a little swollen at the anterior end. It enlarges at the posterior end to form an anterior distinctly developed bulb (*bu<sub>1</sub>*), which in the young mature female is very close to the end bulb, and in the older female almost in the middle of the whole esophagus. Here it is wider than long, while in the young mature female it is longer than wide; size of the anterior bulb in young mature female 85 to 120:80 to 95 $\mu$  (99:86 $\mu$ ); in old female 65 to 80 by 95 to 115 $\mu$  (72.5:105 $\mu$ ). In its interior the spears end. The end bulb (*bu<sub>2</sub>*) is very closely apposed to the first bulb in the young female. It is nearly spherical (size:



115 to 130 by 125 to 145 $\mu$  [125: 134 $\mu$ ]). In the older worm the intermediate section is longer, i. e.,  $\frac{1}{6}$  to  $\frac{1}{4}$  of the total length of the esophagus, and the form of the end bulb is more ellipsoidal, size 150 to 160 by 135 to 140 $\mu$  (155: 137.5 $\mu$ ). In its second half a very well developed valve apparatus is present (Fig. 6, *vaap*). It resembles the capsule of *Papaver rhoeas*. It has the appearance of a circular valve structure, which is connected with the very strongly developed bulb musculature. Apparently the whole formation cares for the trituration of the food. The proportions of structures in the esophagus are as follows: anterior end of the spears, young, mature female 0.59 to 0.79% (0.66%); old, mature female 0.27 to 0.29% (0.28%); first bulb 12.3 to 13.9% (12.9%), resp. 3.3 to 4.2% (3.75%); posterior end of the esophagus 15.4 to 18.2% (16.5%), resp. 6.7 to 7.3% (7.0%) of the body's length.

The spear-apparatus (Fig. 6, 7, *sp*). Three very well-formed spears are present in the esophageal lumen. They are arranged in a triangle, in conformity with the three corners of the mouth opening. Owing to this the lumen of the esophagus has a triangular transverse-section. The anterior end of the spears lies behind the mouth cavity. Instead of closed pointed ends, they have an opening from each canal leading to the exterior. This part which forms the anteriormost end is absolutely smooth. Just behind it a distinct annulation of the spear wall begins. This annulation is in close contact with the annulation of the esophageal lumen. Each annule of the one spear is connected with the annule of the other through an annule of the esophageal lumen (compare Fig. 7). The annulation continues to the posterior end of the spears, which is in the middle of the first bulb. Length of the spears: young, mature female 585 to 690 $\mu$  (652 $\mu$ ) or 11.6 to 14.1% (12.7%) of the total length of the body; old, mature female: 225 to 245 $\mu$  (235 $\mu$ ) or 2.76 to 3.1% (2.98%). There is a fine canal leading from the posterior end of each spear to the muscle apparatus (*bumu*) in the end bulb. This musculature is composed of two muscle systems. One system, moving the valves, consists of two hemispherical parts, which lie on both sides of the esophageal lumen. All muscles of each half proceed from one point and form in toto a spherical calotte. The other system of muscles extends from one point of origin of the muscles attached to the spherical calotte to the origin of the other. This system moves the spear; size of the whole muscle apparatus, 40 to 45 by 30 $\mu$  (42: 30 $\mu$ ).

The cause of symptoms, which lead to the death of the monkey indicate that the whole spear apparatus is movable. The nematodes were found in the rectum in enormous numbers. Two days before the monkey died its feces showed blood. The animal suffered from severe hemorrhages, which were directly responsible for its death. An explanation of this condition can only be found in the fact that the nematodes

attached themselves by suction to the wall of the intestine and then perforated the blood vessels by means of the spears. Along with the hemorrhages there was undoubtedly a toxic absorption by the blood stream of the poison injected through the spears of the nematode into the vessels of the monkey's intestine.

The intestine (*in*) is connected with the esophagus without a cardia. It does not show any unique characters. The rectum is short, about anal width. The tail (Fig. 8) is very long and tapers gradually towards the posterior end. The latter is pointed. No caudal glands could be seen.

There is only one ovary (*ov*), which is very large. It elongates as the female becomes older. In the old females its anterior end is in front of the end bulb of the esophagus. There is a short reflected part which leads into the sex opening. Length of the ovary is 30.9 to 66.4% (50.1%) of the total length of the body. The whole ovary is filled with eggs; there are thousands of these eggs, due to the parasitic life of the nematode (*eg*). The form of the egg (Fig. 12) is an elongated ellipse. Each egg has a double shell which makes it very resistant to external conditions. Size of the eggs 37.5 to 45.0 by 15.0 to 25.0 $\mu$  (41.6: 19.4 $\mu$ ). The larva is unsegmented when the eggs are laid; each cell shows a large nucleus (*nu*). The position of the vulva (Figs. 10, 11, *vu*) is 30.5% of the total body length behind the anterior end. There is a very peculiarly shaped vagina (*va*). Its wall is composed of cells, each of which shows a nucleus. In the interior can be seen a very well developed musculature. There are both a transverse and a longitudinal muscular system. The latter serves to extrude the eggs, the former prevents the return of the eggs into the uterus.

#### DESCRIPTION OF THE MALE

*Size: n = 6*

*L* = 1.140—1.388 (1.238)

*a* = 9.5—14.6 (11.2)

*$\beta$*  = 3.6—4.7 (4.0)

*$\gamma$*  = 39.4—50.4 (46.8)

The *body* is considerably shorter than that of the female. The head, which is not well separated from the other part of the body, tapers gradually towards the anterior end, which is  $\frac{1}{3}$  to  $\frac{1}{4}$  of the greatest body width. The posterior end is short and rounded. The *cuticle* consists of several layers. The annulation begins in front of the mouth cavity and continues to the anus. The cuticula has a thickness of 2.5 to 7.5 $\mu$  (5 $\mu$ ). The musculature consists of the same muscle systems as in the female, but these systems are not so well developed, except the preanal musculature, which serves the copulation organs (Figs. 16, 17).

The head (Figs. 13, 14) is rounded and truncated. There are three lips with a well developed muscle system. The mouth cavity is larger than that of the female, i. e., about  $\frac{1}{3}$  of the corresponding body width or 10.5 to 12.5 by  $7.5\mu$  ( $11.5:7\mu$ ). The cuticular skeleton is not so distinctly developed as in the female. Apparently there are no reinforcements for the spears. The amphids are present, have the same form as in the female, but are relatively larger,  $\frac{1}{11}$  to  $\frac{1}{12}$  of the corresponding width or 2 to  $2.5\mu$ .

The esophagus (Fig. 15) has the same structure as that of the female. The sizes of the different parts are as follows: anterior bulb, 35 to 40 by 35 to  $40\mu$  ( $37.5:36.9\mu$ ); end bulb, 50 to 60 by 50 to  $65\mu$  ( $54.5:59\mu$ ). The valve apparatus is also present, but is almost completely covered with the musculature. Size of the muscle apparatus in the end bulb is 20 to 30 by 20 to  $30\mu$  ( $27.5:22.5\mu$ ). In the esophageal lumen the three spears are visible. Their relative length is a little more than in the female, i. e., 18.5 to 20.8% (19.6%) of the total length of the body or 230 to  $275\mu$  ( $254\mu$ ). The proportions in the esophagus are as follows: anterior end of the esophagus, 0.8 to 1.0% (0.9%); first bulb, 16.0 to 21.7% (19.5%); posterior end of the esophagus, 21.9 to 28.6% (25.9%) of the total body length. The intestine is without any differential characteristics. The tail is very short and rounded. It is transformed to a sex organ and has lost its function as an organ of locomotion. There is one long extended testis, which has a reflected anterior end (Fig. 16, *terf*). The reflected part has a length of 85 to  $195\mu$  ( $115\mu$ ) or 6.8 to 11.5% (8.3%) of the total length of the body. The total length of the whole testis is 54.3 to 55.9% (55.2%) of the total body length. There is only one spiculum (*spi*), which is 40 to  $45\mu$  ( $41.1\mu$ ) long. It resembles a sword. Its anterior end is slightly swollen at the end. The middle part is enlarged. The posterior part tapers gradually to a rounded point. In the ventral view the spiculum is similar to a sternum. There is no gubernaculum present. However, there can be seen a gubernaculum-like formation just in front of the anus (Fig. 18, *gub?*), which seems to be a tubiform organ, through which the spiculum glides. It is possible that it is a reinforcement of the end part of the wall of the testis.

The anal armament is typical. The formation of the cuticula in the ventral view (Fig. 18) is very similar to a bursa. It shows two pairs of muscles (*mu*), which serve for the contraction of the papillae. Four pairs of papillae are present (Figs. 17, 18, *pa* 1-4). The first pair is preanal, just in front of the anus; the second pair can be seen on either side of the anus and is largest in size. Just behind this pair lies the third pair of papillae. At the base of the spine the fourth pair is inserted. Each papilla has an opening to the outside. The papillae serve for fixation of the male during copulation.

The spine (*s*), which has a length of 15 to 20 $\mu$  (16.5 $\mu$ ), is terminal. It has an inner canal, which is seen very distinctively in the ventral view (*sca*). One may conclude, that the spine probably has an excretory pore, which could not be found due to the small size of the organ. The proportion at the posterior end is as follows: spiculum: 1; *pa*<sub>1</sub>—anus: 0.16; *pa*<sub>3</sub>—anus: 0.12; *pa*<sub>4</sub>—anus: 0.4.

#### DISCUSSION

A comparison with other oxyurid species shows that the posterior end of the male has analogies to *Enterobius atelis* Cameron (1929), as well as it is possible to judge from the rather diagrammatic drawings accompanying Cameron's description. The arrangement of the papillae differs from that of *E. legothricis* Buckley and *E. duplicidens* Buckley (1931) where three pairs of the papillae are anterior to the posterior end of the spiculum. There is also a difference in the formation of the spiculum, which does not show the anterior appendices described by Buckley. From *E. vermicularis* (Travassos, 1925) the present form differs in the formation of the male's caudal end, where the spiculum seems to be very long. However, the most distinct difference from the species cited is the formation of the head. The three spears, the very distinctly developed anterior bulb, the tri-partite mouth cavity, the whole muscle system in the end bulb—all these are typical characteristics of the present species, which are not present in the species cited. A comparison with human *Enterobius* material showed that the spears are absent in this material. It is also noteworthy that the female has a long unpaired reflected ovary, while in the known oxyurid species the female sex organs are paired.

When one examines the living nematodes, one can see that in all specimens the head has the same type of cuticula as the rest of the body. The formations of the anterior end, which are partly given by Cameron (1929, Figs. 24, 25, 17, 18) and Buckley (1931) are caused by the preservation of the specimens. In abundant material which the writer preserved in a solution of hot formaldehyde (4%) and 10% glacial acetic acid, many specimens show this contraction, which caused the projecting of the head end.

#### PATHOLOGY

The domicile of the parasite is the large bowel, where it was found in enormous numbers. According to information which I have been able to gather it appears that this is the first time that infection with a species of the family *Oxyuroidea* has contributed to the death of a monkey. It seems practically certain that the monkey acquired this *Oxyuronema*-infection first in Panama. The resistance of the host to the parasite gradually weakened, which argues for the possibility of the rapid reinfection with the nematode in the last days of the monkey's life.

The signs and symptoms attributed to the infestation were hemorrhage and probably toxemia, leading to the death of the animal. That the infection ended fatally was presumably due to the number of the parasites, which became increasingly more numerous. The pathogenicity of *Oxyuronema* is due, no doubt, to the presence of the spears, which traumatize the rectal mucosa and allow the introduction of toxins and micro-organisms into the blood-stream; also to the presence of the enormous quantity of eggs, which matured very rapidly under favorable conditions such as are found here in New Orleans.

## REFERENCES

- Buckley, J. J. C. 1931.—On Two New Species of *Enterobius* from the Monkey *Lagothrix humboldtii*. Jour. Helm., 9:133-140.  
 Cameron, T. W. M. 1929.—The Species of *Enterobius* Leach, in Primates, Jour. Helm., 7:161-182.  
 Travassos, L. 1925.—Revisao do genero *Enterobius* Leach 1853. Mus. Nac. Rio de Janeiro, n. e. No. 2, 11 pp., 2 pl.

## DESCRIPTION OF PLATES XXVI AND XXVII

The scale in figures 1, 2, 8, 9, and 10 equals 0.10 mm.; in figs. 1a, b, c, d, 5, 6, 7, 12, 13, 14 and 19 equals 0.01 mm.; in all other figures it equals 0.03 mm.

- Fig. 1.—Anterior end of body of mature female.  
 Fig. 1a.—Annulation of cuticula at head end; b, annulation of cuticula behind head; c, annulation in middle of body.  
 Fig. 2.—Anterior end of body of old mature female.  
 Fig. 3.—Head of female.  
 Fig. 4.—End view of head of female.  
 Fig. 5.—Amphid of female.  
 Fig. 6.—Muscle system in end bulb of female.  
 Fig. 7.—Construction of spears in female.  
 Fig. 8.—Posterior end of body of female; d, cuticula at end of tail.  
 Fig. 9.—Lateral view of anus of female.  
 Fig. 10.—Vulva.  
 Fig. 11.—Musculature of the vulva.  
 Fig. 12.—Egg.  
 Fig. 13.—Head of male.  
 Fig. 14.—Amphids of male.  
 Fig. 15.—Posterior end of esophagus of male.  
 Fig. 16.—Anterior end of testis.  
 Fig. 17.—Posterior end of body of male; lateral view.  
 Fig. 18.—Posterior end of male; ventral view.  
 Fig. 19.—Spine.

## ABBREVIATIONS

*am*, amphid; *an*, anus; *anmu*, anal musculature; *bu*, bulb; *bumu*, bulb musculature; *bur*, bursa; *eg*, egg; *gu*, gubernaculum; *in*, intestine; *li*, lip; *limu*, lip musculature; *mc*, mouth cavity; *mcsk*, mouth cavity skeleton; *mu*, muscles; *nu*, nucleus; *oe*, esophagus; *oelu*, esophageal lumen; *ov*, ovary; *pa*, papilla; *s*, spine; *sca*, spine canal; *sp*, spear; *spi*, spiculum; *tamu*, tail musculature; *te*, testis; *terf*, testis reflection; *va*, vagina; *vaap*, valve apparatus; *vamu*, vaginal musculature; *vu*, vulva.



KRIES—NEW PATHOGENIC NEMATODE

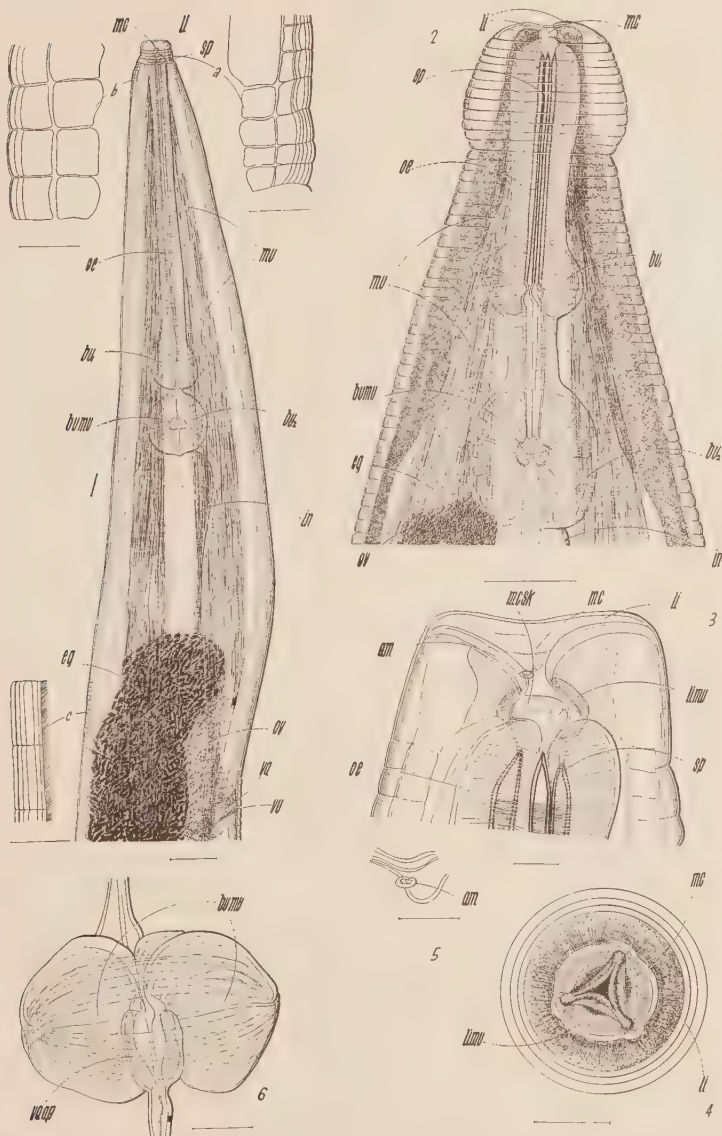


PLATE XXVI

KRIES—NEW PATHOGENIC NEMATODE

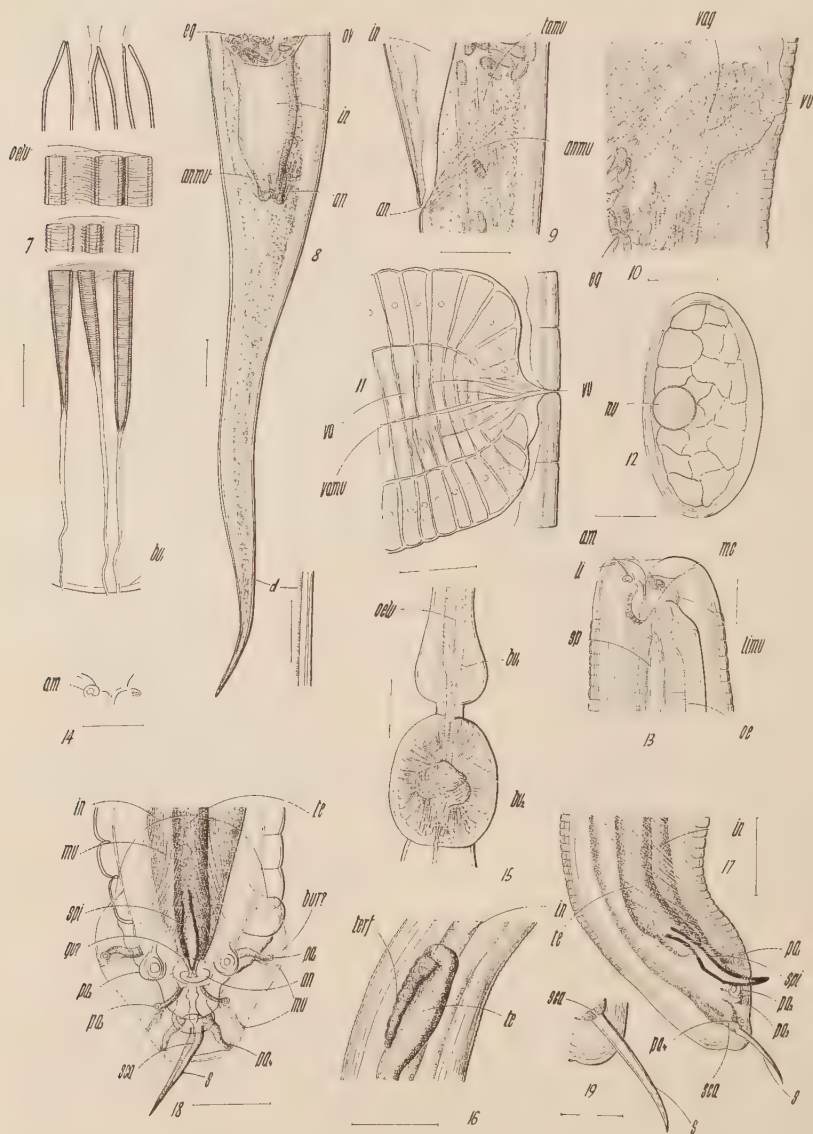


PLATE XXVII

## SOCIETY PROCEEDINGS

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### HELMINTHOLOGICAL SOCIETY OF WASHINGTON

#### *One Hundred Fortieth to One Hundred Forty-Third Meetings*

The one hundred fortieth meeting was held September 19, 1931.

B. G. Chitwood presented a note on variation of the excretory system of nematodes.

New host records for *Dispharynx spiralis* by E. B. Cram.—In an English sparrow, *Passer domesticus domesticus*, caught in Washington in March, 1931, were found 1 female and 3 male specimens of *Dispharynx spiralis*. In August, 1931, F. R. Beaudette submitted specimens of this nematode collected from a young robin, *Planesticus migratorius migratorius*, in New Jersey. Fifty-four specimens of *D. spiralis* were present in the proventriculus, in addition to four other kinds of parasites in other locations. These two records of members of the Passeriformes enlarge considerably the range of bird hosts for this nematode in this country; gallinaceous game birds are most frequently recorded in this connection, with domestic Galliformes and domestic Columbiformes as occasional hosts.

*Nematodirella longispiculata* from the moose, *Alces americanus americanus*, by G. Dikmans.—In April, 1931, the Zoological Division received from Dr. R. O. Christensen of the University of Minnesota some nematodes collected from a moose at Winton, Minnesota. These nematodes were identified as *Nematodirella longispiculata*. This nematode was first reported by Romanovitch in 1915 as a parasite of the reindeer from the Archangel district in Russia under the name of *Microcephalus longissime spiculatus*. An examination of the reindeer nematodes collected by Dr. S. Hadwen in Alaska, which are part of the collection of parasitic nematodes of the Bureau of Animal Industry, showed that this nematode is also found in reindeer in Alaska. This is the first report of its occurrence in the continental United States in a typically American ruminant.

H. E. Ewing presented a note on young toads as hosts of chiggers.

Control of liver fluke in California by Robert Jay.—About three years ago work was begun by the Bureau of Animal Industry in cooperation with the sheep growers and various officials of the state of California on the control of liver flukes in sheep in that state. The sheep were treated with carbon tetrachloride and the snail intermediate hosts of the fluke were destroyed by means of copper sulphate or their breeding places drained, filled in or fenced away from sheep. Within three years flukes in sheep in California have been practically eradicated as judged by meat inspection reports and the reports of sheepmen over the state. This does not mean that no sheep in California have liver flukes, but does mean that most of the flocks are free and that as a source of loss, flukes are under control over practically the entire state. The sheep industry has saved large sums of money that would have otherwise been lost from deaths of sheep and from diminished mutton and wool production.

Transmission of anaplasmosis by *Dermacentor variabilis* by C. W. Rees.—Anaplasmosis has now been experimentally transmitted to four bulls by means of the tick, *Dermacentor variabilis*, the last two cases being nymph to adult transmission. These experiments have established these facts: Larvae of *D. variabilis* may engorge on infected bovines and transmit anaplasmosis as nymphs to susceptible bovines; nymphs of this tick may engorge on infected bovines and transmit anaplasmosis as adults to susceptible bovines. Transmission from adults engorging on infected bovines through the egg to larvae engorging on susceptible bovines has not yet been accomplished.

M. C. Hall presented the following note: In August, 1931, five peccaries, *Pecari angulatus angulatus* (Cope), killed on the King Ranch, Kingsville, Texas,

were examined post mortem. No endoparasites were found. Large numbers of ticks and fleas were collected from the peccaries.

A. McIntosh reported on the identification of the ticks mentioned above. The collection consisted of 285 specimens and represented the following species: *Amblyomma cajennense*, 167 males, 46 females, and 53 nymphs; *Dermacentor variabilis*, 7 males; and a species of *Dermacentor* regarded as new, represented by 8 males and 4 females. A description of this species will be published elsewhere. This is the first record in which a member of the genus *Dermacentor* has been reported from the peccary.

Bot larvae in tongue of the rabbit and horse by E. E. Wehr.—A young Belgian hare was fed 300 larvae of *Gasterophilus intestinalis* taken from eggs clipped from the legs of horses. The larvae were mixed with a little water and placed directly on the tongue of the animal by means of a medicine dropper. This number of eggs was fed over a period covering a week, one half of the larvae, however, being fed on the last day. This was on Friday. On the following Sunday and Monday the rabbit was very sick, both water and food being refused. This temporary disorder might have been associated with the burrowing of the young bot larvae into the tissue of the tongue. On the 13th day following the first dose of larvae, the rabbit was killed and the stomach, esophagus, trachea, mouth cavity, cheek muscles, lips and tongue were examined for the presence of bots. No larvae were found except in the tongue. A pressed section of the tongue revealed the presence of a large number of larvae of *G. intestinalis* similar in appearance to the newly hatched larvae except larger in size. As nearly as one could determine, the larvae were embedded just beneath the corium layer of the mucous membrane. Recently the stomach, esophagus, throat and tongue of a freshly killed horse were examined. Five young bot larvae similar in appearance to those found in the tongue of the rabbit, but slightly larger in size, were found embedded just below the thick epidermal layer covering the dorsal surface of the tongue.

The one hundred forty-first meeting was held October 16, 1931.

N. A. Cobb presented the following notes:

(1) On the use of the nema, *Metoncholaimus pristiurus*, in the teaching of biology. (2) The biology of the group of Epsilonema-like nemas. (3) On the finding of the type species *Enoplolaimus vulgaris* de Man, on this side of the Atlantic on the south shore of Martha's Vineyard. (4) A key to the nema genera.

Kamala as an anthelmintic for removing bothriocephalids from pelicans by M. C. Hall.—In 1930, 6 pelicans were treated for tapeworms, as follows: Four pelicans were given a level teaspoonful, equivalent to about 1 dram or 4 grams, of kamala, washed down with 2 ounces of water. One bird passed one tapeworm, *Diphyllobothrium cordiceps* (= *Dibothrium cordiceps*). On post-mortem examination all birds were free from tapeworms. Two pelicans were given one trout each, the trout in one case being gutted and a level teaspoonful of kamala being put into slits cut in the flesh from the body cavity towards the skin. The other trout was not gutted and the kamala was put into the body cavity through a slit cut in the abdominal wall. The first of these pelicans passed a tapeworm, *D. cordiceps*. Both pelicans were free from tapeworms on post-mortem. While only two of the birds harbored tapeworms, the removal of these worms and the freedom of all treated birds from tapeworms post-mortem, indicates that kamala is probably dependably effective for the removal of bothriocephalid tapeworms from pelicans, when used in the dose of about 1 gram. These findings are in agreement with the fact that kamala is the most effective of the known anthelmintics for removing tapeworms from birds and with the theoretical likelihood that bothriocephalid tapeworms, with relatively large heads and the relatively weak attachment of two shallow suckers and no hooks, would be comparatively easy to remove.

The development of *Diphyllobothrium cordiceps* (= *Dibothrium cordiceps*) in *Pelecanus erythrorhynchus*, by Lowell Woodbury.—The following experiment in connection with the life history of *D. cordiceps* was outlined by Dr. M. C. Hall and carried out by the writer during the summer of 1930. A young pelican was treated with 1 gram of kamala in order to remove any specimens of *D. cordiceps*



that it might harbor. No worms were passed and fecal examination did not show any tapeworm eggs present. Specimens of the Yellowstone trout, *Salmo lewisi*, infected with the larvae of *D. cordiceps*, and a few other species of trout, and grayling, not infected, were fed to the pelican as follows:

August 14, one trout with two free larvae in stomach region and many encysted larvae on stomach and pyloric ceca; August 15, one trout apparently free from larvae; August 16, one trout with a number of larvae; August 17, one trout with a large number of larvae; August 18, one trout with 14 larvae; August 20, one trout with approximately 15 larvae and one trout not examined for parasites; August 22, one trout with about 15 larvae; August 25, one trout with about 20 larvae; August 26, one trout with 2 large larvae, about 4 inches long, in ovaries; August 27, one trout apparently free from larvae; August 29, one trout not examined for parasites; August 30, one trout and one grayling, both free from larvae; September 1, one trout with about 5 larvae and 2 graylings, free from larvae; September 3, one trout apparently free from larvae; September 4, two trout apparently free from larvae; September 5, one grayling and one trout free from larvae; September 6, two trout, both free from larvae; September 8, one trout and 3 graylings free from larvae.

On September 10, the pelican was killed and a postmortem examination showed the presence of 2 large and one small tapeworm which were identified by Miss Myrna F. Jones of the Zoological Division, Bureau of Animal Industry, as *D. cordiceps*. The very small number of tapeworms obtained from this feeding experiment indicates that the large majority of the larvae fed were unable to develop and suggests that the smaller larvae may not be able to develop to adults in the primary host. The presence of two fully developed adults within less than a month from the time the experiment began indicates that the tapeworm is capable of maturing in less than one month.

Benjamin Schwartz presented a note correcting the spelling of *Oesophagostomum maplestoni* to *Oesophagostomum maplestonei*.

L. A. Spindler presented a note on the nodules due to larvae of *Oesophagostomum longicaudum*.

The following notes were given by G. Steiner. (1) On the occurrence of the sugar beet nema, *Heterodera schachtii*, on *Polygonum pennsylvanicum* and *Polygonum punctatum*, two new hosts from this country. (2) A new species of *Deontostoma* from the coast of California.

The one hundred forty-second meeting was held at the School of Hygiene and Public Health, Baltimore, Md., November 20, 1931.

The following membership policy was adopted: There shall be three classes of members, viz.: (a) Active, (b) Foreign, and (c) Honorary.

(a) Active members. Any person interested in parasitology or helminthology may become a member of the society upon nomination and election. The dues shall be two dollars (\$2.00) per year if the member desires the proceedings, or one dollar (\$1.00) per year if the member does not desire the proceedings.

(b) Foreign members. A limited number of foreign parasitologists and helminthologists may become members of the society upon application and election. The dues of these members shall be the same as for the active members.

(c) Honorary members. Honorary members may be elected upon nomination by the Committee on Honorary Membership. Such members shall receive the proceedings of the society.

All classes of members may present short notes dealing with some phase of parasitology or helminthology to be read before the society and to be published in its proceedings.

All money accruing from dues or from other sources which is in excess of that necessary for the expenses of the society shall be transferred to the Ransom Memorial Fund to become a part of the principal of said fund. Any members in arrears for dues for two years may be dropped from the rolls of the society without notice.



Cephalic sensory organs of *Ascaridia lineata* and a comparison with those of oxyurids, by B. G. Chitwood and J. E. Alicata.—Ackert has recently described and figured the cephalic sensory organs of *Ascaridia lineata* concerning which he states: "The oral opening is surrounded by three prominent lips, one dorsal and two sub-ventral, each of whose distal margins is divided into three lobes; one median and two lateral. . . . Two conspicuous papillae occur on the dorsal lip and one on each of the subventral lips." Schwartz, on the other hand states: "Each lip bears two papillae, these having also been observed by Lane."

An examination of the heads of several specimens of *Ascaridia* both in section and *en face* view shows that the dorsal lip bears two large submedian duplex papillae while each of the subventral lips bear a large submedian duplex papilla, a smaller simple lateral papilla, and an amphid. The structure of the papillae as well as their number and distribution is especially interesting from the point of view of the relationships of the ascarids and suckered oxyurids. As Goldschmidt has so plainly shown many years ago, *Ascaris* also has these large duplex submedian papillae, the smaller, simple lateral papillae and the amphids. However, an examination of the head of *Heterakis gallinae* shows that it likewise has the above mentioned characters. Thus it appears that the position of *Ascaridia* hinges on the relative importance of the loss of the valves in the esophageal bulb as against the presence of a preloacal sucker in the male. It is questionable whether the presence or absence of valves in the esophageal bulb is of superfamily rank as would be indicated by the placing of *Ascaridia* in the Ascarioidea. Since the muscle distribution in the esophagus is the same as would be present in the esophagus of a nematode with a valvular bulb the point appears of less significance.

In the same connection it is interesting to consider the head of *Cissophyllus roseus*, one of the most highly ornamented in the group of suckered nematodes. As in the previously mentioned cases there are four large submedian duplex papillae, two simple lateral papillae, and amphids. Upon first glance the similarity ceases at that point. However, a more careful consideration shows that the cephalic organs are actually in three groups, a dorsal and two subventral. The two subventral lips are more highly developed while the dorsal lip is somewhat reduced.

In *Subulura distans* there are three small lips which are reduced and do not form the anterior end of the body as in the other forms previously mentioned. Four large submedian duplex papillae are present, but the innervations lie one directly anterior to the other so that they are very difficult to distinguish except in longitudinal section. The amphids and lateral papillae likewise lie in tandem, the lateral papillae being situated immediately anterior to the amphids. The *en face* view shows only the amphid since its tube is much more conspicuous than the delicate innervation of the lateral papilla. Thus in the case of these four suckered nematodes, now placed in four families, in two superfamilies and orders, the heads have been found to be essentially the same. Thus far the one-spiculed, non-suckered oxyurids on the other hand, have been found to agree among themselves and differ from the former group in that the lateral papillae are absent. The four submedian papillae are simple, not duplex in the Oxyuridae, while in the Thelastomidae there are eight submedian simple papillae.

Oxyurid parasites of Blattidae by B. G. Chitwood.—A study of the parasites of Blattidae and Myriapoda has made it necessary to restudy the oxyurid parasites of these groups which are present in the Leidy Collection. Walton (1927) states: "A large number of Oxyurid forms apparently from cockroach hosts and representing at least three species of parasites were found in an unlabelled vial." He identifies them as *Aorurus diesingi* (Hammerschmidt, 1838), *Aorurus (Thelastoma) bulhõesi* (Magalhães, 1900) and *Isakis robusta*, new species. These specimens have recently been examined through the courtesy of Prof. J. Percy Moore of the University of Pennsylvania and reidentified as *Aorurus agile* Leidy, 1849 [syn. *Aorurus diesingi* (Hammerschmidt, 1838) of Walton, 1927]; *Thelastoma attenuatum* Leidy, and *Thelastoma spicatum* Cobb, 1920 [syn. *Thelastoma bulhõesi* (Magalhães, 1900) of Walton, 1927]; and *Rhigonema infecta* (Leidy, 1853) (syn. *Isakis robusta* Walton, 1927). These are all parasites of *Spirobolus marginatus* and probably represent the types of the three species originally described

from that host by Leidy. The specimens agree in all particulars with parasites collected by the author from *Spirobolus marginatus*.

The occurrence of *Uncinaria stenocephala* from the Fisher, *Mustela* sp. by B. G. Chitwood.—The material consisting of several specimens was sent for identification to the Zoological Division, Bureau of Animal Industry, by R. L. Conklin, MacDonald College, Quebec, Canada. This is apparently the first report of the occurrence of *Uncinaria stenocephala* in a member of the genus *Mustela*.

B. G. Chitwood also presented some studies by Mrs. Chitwood and himself on the histology of *Scarabanema cylindrica*.

G. Steiner presented a note on a new filaroid parasite of *Bdellostoma hep- tatrema* J. Müll. from the Cape of Good Hope.

Additional notes on intermediate hosts of poultry tapeworms by Myrna Jones.—Cysticercoids of *Railletina magnimunda*, a tapeworm of guinea fowl, have been found in the beetles *Aphodius granarius* and *Amara (Amara) fallax* after these beetles were fed with gravid segments of the tapeworm. As many as 50 cysts were present in one instance. Most of the material was fed to chickens with negative results. Thus far, all attempts to infect chickens and turkeys with this tapeworm from guinea fowl have been unsuccessful. Cysticercoids of *Railletina cesticillus* were found as natural infestations in the beetles *Stenolophus conjunctus* (numerous cysts) and *Stenocellus debilipes* (18 to 20 cysts). In both instances young chickens fed the material harbored specimens of *R. cesticillus* upon postmortem examination. Specimens of *Stenolophus conjunctus* were also infected with cysts of *R. cesticillus* by experimental feedings with this species of tapeworm.

After experimental feedings the following additional beetles contained cysticercoids of *R. cesticillus* when dissected. *Stenocellus rupestris* dissected after 19 days; 6 cysts obtained and fed to a young chicken kept under controlled conditions; upon examination of the chicken after 31 days, one adult specimen of *R. cesticillus* was obtained; *Aphodius* sp., material not fed to chickens; *Tenebrio* sp., only 2 cysts, material not fed to chickens; *Harpalus nitidulus*, 5 to 6 cysts present, fed to a young chicken which was negative for helminths upon postmortem examination. A guinea fowl was infected with 2 specimens of *Railletina cesticillus* after having been fed cysts of that species from *Cratacanthus dubius*. The cysts were undoubtedly of chicken origin. In a previous report of a transfer of this species of tapeworm from chickens to guinea fowl there was recognized a possibility of a natural infestation of material from guinea fowl in the beetle used.

Natural infestations of *Hymenolepis carioca* in 2 specimens of *Aphodius granarius* have been noted, with at least 80 cysts present in one beetle and between 200 and 250 cysts in the other. The specimens of *Aphodius* were collected from a poultry yard near Beltsville, Maryland. In addition, 3 specimens of *Choeridium histeroides*, collected from the same region, were found to be carrying heavy natural infestations of *Hymenolepis carioca*. In one instance, about half of the material was fed to a chicken from which approximately 50 mature specimens of *H. carioca* were obtained upon postmortem examination. All but ten cysts from a second beetle were fed to a young chicken from which at least 150 (estimated number) mature specimens of *H. carioca* were obtained upon postmortem examination. The author is indebted to Dr. E. A. Chapin and Dr. L. I. Buchanan for their kindness in identifying the beetles used in this work.

G. Winfield presented a note on the immunity of snails infested with the sporocysts of the strigeid, *Cotylurus flabelliformis*, to the penetration of its cercariae.

Observations on two ciliates, a species of *Balantidium* and a species of *Troglo-dytella* from the chimpanzee by E. C. Nelson.—*Balantidia* from a culture containing no starch were found to ingest starch with such eagerness and rapidity that large individuals had engorged themselves within four minutes after the starch had been added. When this was observed under the microscope apparently no food vacuoles were formed, the starch passing directly into the endoplasm from the cystopharynx. For better observation rice starch stained purple with iodine and washed was fed and found to be eagerly eaten although carmine granules had been refused. The progress of these starch granules was clearly observed and confirmed the observa-

tion that apparently no food vacuoles were formed. The species of *Troglodytella* from the Chimpanzee was found to live and multiply in a *Balantidium* culture medium for a period of seven days.

Frederic Fish presented a note on the high incidence of ciliates in fish from the Gulf of Maine.

Deficient diet as a factor rendering dogs susceptible to the cat strain (Scott) of *Ancylostoma caninum* by A. O. Foster and W. W. Cort.—It was found that dogs on a deficient diet were much more susceptible to infection with the cat strain of *A. caninum* than those on a good diet. In five old dogs on the deficient diet the average development to adult worms was 3.12 per cent of the infective larvae given, while in five control puppies on a good diet the development was only 0.025 per cent.

Development outside of the human body and excystation in monkeys of cysts of *Endamoeba histolytica* by Robert Hegner.—Cysts when passed by carriers often contain only one or two nuclei instead of the four nuclei characteristic of mature cysts. Studies of preparations made from a single stool at intervals during 29 hours resulted in the finding of an increase in binucleate and quadrinucleate cysts and a decrease in uninucleate cysts, thus proving that development continued within the cysts after they were passed. Cysts were injected into the stomach of three brown howler monkeys and the monkeys were killed one hour and twenty minutes, one hour and thirty minutes and three hours later, respectively. It was found that most of the cysts hatch in the small intestine in about three hours. The process of excystation is similar to that described in the literature in other species of amoebae. One amoeba escapes from the cyst wall. Binucleate and uninucleate as well as quadrinucleate (mature) cysts excyst, hence cysts do not need to be mature to be infective.

Another case of the transmission of anaplasmosis by *Rhipicephalus sanguineus* by C. W. Rees.—A second experiment resulting in the successful transmission of anaplasmosis by *Rhipicephalus sanguineus* was carried out as follows: A heifer 10 months old was splenectomized on Aug. 26, 1931, and by failing to develop a case of anaplasmosis subsequent to the splenectomy was thereby established as a susceptible animal. Blood smears taken daily for 17 days and once or twice a week for several weeks thereafter were negative for anaplasmosis. About 70 nymphs of *R. sanguineus*, which had engorged as larvae on a clinical case of anaplasmosis following exposure to infective nymphs of *Dermacentor variabilis*, were put in bloomers on this heifer on September 30, 1931, and 22 engorged nymphs were removed on October 5 and 6. This animal developed anaplasmosis on November 3. The incubation period was, therefore, not more than 34 days. On November 4, the heifer refused food and water; on November 5, about 50 per cent of erythrocytes contained Anaplasma; on November 9, the heifer died. This is the second successful transmission of this disease with this tick out of four experiments; in both cases a splenectomized animal was used and both cases were fatal. It will be noted that the incubation period, 34 days, is in agreement with the period of approximately one month in which five other cases of successful tick transmission of the disease at Jeanerette, La., has been established.

The life history of *Gasterophilus intestinalis* by E. E. Wehr.—A two-and-a-half months old colt was led to a total of 12,919 larvae of *Gasterophilus intestinalis* from July 27 to October 20, 1931. The larvae were fed on 70 of the days in this period of time and the average number fed per day was about 185. The larvae were removed from the eggs, placed in water in a watch glass, and dropped on the tongue by means of a medicine dropper. The colt was killed on October 22, and on postmortem examination several small larvae were found just beneath the epidermis of the cheeks and lips. A large number of larvae, from 1.5 to 6 mm. long, were found under the mucous membrane of the tongue; one larva about 6 mm. long was found crawling over the dorsal surface of the tongue near the pharynx; one larva about 6 mm. long was found attached to the mucous lining of the esophagus, about midway between the pharynx and stomach; and 713 larvae, from 6 mm. long to about two-thirds grown, were found in the esophageal portion of

the stomach. Of the larvae found in the tongue, the smaller ones were usually in the anterior portion and the larger ones in the posterior portion. Apparently when the larvae of *Gasterophilus intestinalis* get into the mouth of a horse they usually invade the tongue and, judging by excavations found under the mucosa, they wander about to a certain extent and after reaching a length of about 6 mm., they leave the tongue and either migrate actively down the esophagus or are swallowed and then attach to the lining of the stomach, where they continue to develop. A detailed account with notes on pathology will be published later.

The naming of larval trematodes by M. C. Hall.—An attempt to index the literature dealing with larval trematodes puts the indexer in a very embarrassing situation in trying to handle the polymorphic nomenclature of this group. Part of the names follow the binominal nomenclature, but a great many names are in arbitrary form and some of these forms cannot be fitted into an alphabetical sequence. As illustrations we note that one writer has a series running Cercaria 1, Cercaria 2, etc. Another has the series Cercaria I, Cercaria II, etc. Another has the series Cercaria X.1, Cercaria X.2, etc. Another has the series Cercaria helvetica I, Cercaria helvetica II, etc. Another has the series Cercaria nicobarica I, Cercaria nicobarica II, etc. Another has the series Cercaria A, Cercaria B, etc. Added to this are the numerous cases where a cercaria is reported but not designated by name or designated as *Cercaria* species. There are also such names as *Cercaria Limnaea truncatula*. We are also having Furcocercaria 1, Furcocercaria 2, etc., Lophocercaria A, Lophocercaria B, etc., and Xiphidocercaria A, Xiphidocercaria B, etc. It is obvious that a continuation of these systems will, in a very short time, require that in any reference to these names one quote the author and date in order to separate the A, B, C of one man from the A, B, C of another, and the 1, 2, 3 of one man from the 1, 2, 3 of another, and that this will be a burdensome thing. It is also obvious that in an alphabetical index it will be very difficult to incorporate Cercaria 1 and Cercaria I with anything more than an arbitrary basis for the sequence. There appears to be no reason why all cercariae should not be given a binominal name, as most of them have been already. This would simplify the business of referring to them and if the names proved to be synonyms they could be dropped into synonymy with very little effort. As matters stand the nomenclature of larval trematodes is rapidly becoming a mess and if the workers on nematodes and cestodes should adopt similar procedures in nomenclature the literature of parasitology would become a much less dignified and useful thing than it is at present.

On the genera *Centrocestus* Looss and *Stamnosoma* Tanabe by E. W. Price.—In connection with a review of the heterophyid trematodes infesting domesticated carnivores the writer has been confronted with the question of the validity of the genus *Stamnosoma* Tanabe. Several writers have pointed out the similarity of *Stamnosoma* to *Centrocestus*, but up to the present time the question of their identity has been left unsettled. Chapin (1926) pointed out that "*Stamnosoma* Tanabe, 1922, appears to be inseparable from *Centrocestus* Looss, 1899," and Stiles and Hassall (1926) also are of this opinion. Faust and Nishigori (1926) believe that the two genera should be retained since *Stamnosoma* has a well developed esophagus, which *Centrocestus* does not possess, and because Looss' figure (1896, fig. 65) of *Distomum cuspidatum*, type species of the genus *Centrocestus*, "shows definite prepharyngeal outpocketings, which *Stamnosoma* does not possess." Several other minor differences between the two genera were noted. As regards the "prepharyngeal outpocketings," which Faust and Nishigori regarded as especially important, a comparison of figure 64 with figure 65 (both appearing in Plate VII of Looss' monograph "*Recherches sur la faune parasitaire de l'Egypte*") shows definitely that no such structures exist, the apparent outpocketings being due to a shortening and widening of the prepharynx as a result of contraction of the anterior end of the body. A point by point comparison of the species referred to the two genera has convinced the writer that such differences as exist between them are of only specific value and that *Stamnosoma* must fall as a synonym of *Centrocestus*; *Stamnosoma armatum* Tanabe and *S. formosanum* Goto (in Nishigori), therefore, become *Centrocestus armatus* (Tanabe) and *C. formosanus* (Goto) respectively.



The probable type host of *Braunotrema pulvinata* (Braun) by E. W. Price.—Recently a number of trematodes were collected by B. G. Chitwood from the intestine of a specimen of *Podocnemis expansus* which died in the National Zoological Park, Washington, D. C. Among these were found a number of specimens of *Braunotrema pulvinatum* (Braun) [Syns. *Distomum pulvinatum* Braun = *Thaumatoctyle pulvinata* (Braun)]. An examination of the trematodes from turtles in the helminthological collection of the U. S. National Museum also showed that a few specimens of this fluke had been collected from *P. expansus* in 1901, by A. Hassall, the entry in the catalogue stating that the turtle was from Brazil. In view of the fact that the specimens upon which Braun based his description of this fluke had been collected from fluviatile turtles (scientific names not given) by Natterer in Brazil, it appears reasonable to assume that *Podocnemis expansus* was probably the type host of this trematode.

Additional observations on bird hosts of *Dispharynx spiralis* by E. B. Cram.—A robin from New Jersey and a sparrow from the District of Columbia were reported in these proceedings as hosts of *Dispharynx spiralis*. In looking through the collection of the Zoological Division, there were noted specimens sent in from Riverton, N. J., in 1920, collected from a catbird, *Dumetella carolinensis*; these specimens were identified as *Dispharynx* species at that time by M. C. Hall. The author has identified them specifically as *D. spiralis*. Soon after this finding, there were received from C. B. Hudson of New Brunswick, N. J., specimens of *Dispharynx* which appear also to be *D. spiralis*; these also originated from a catbird, 230 specimens being said to be present. There are therefore two cases from New Jersey, of this passeriform bird serving as host for this nematode. Experiments to investigate the interrelationship of this species of parasite in wild and in domestic birds have recently resulted in artificial infection of a guinea fowl, *Numida meleagris*, with *D. spiralis*. Isopods (pill bugs and sow bugs) are being used as intermediate hosts in these experiments. *D. spiralis* collected from a bobwhite quail was transmitted to domestic pigeons and from them to the guinea fowl.

Pathology of experimental amoebiasis in monkeys by C. M. Johnson.—During the past summer the author along with R. W. Hegner and R. M. Stabler, conducted a series of cross-infection experiments with *Endamoeba histolytica* using monkeys as the experimental hosts. A series of 24 monkeys, including six species, were inoculated with the infective organism of which 12, including 3 species, became infected and showed definite pathological lesions. Material for infection was obtained from two sources, trophozoites from acute cases of human amoebiasis and cysts from carrier cases. Of the 12 animals infected, seven were inoculated with trophozoites per rectum and five with cysts per os. Histological examination of the lesions revealed a picture very similar to that described for human amoebiasis. This was found to be especially true of the monkeys infected with trophozoites from human cases. The cyst monkey showed a somewhat different type of pathology which was considered the very earliest manifestation of the disease. Examination of freshly fixed and postmortem tissue revealed the fact that amoebae wander about in the tissue after the death of the host but cause no injury or necrosis of the tissue.

The one hundred forty-third meeting was held December 19, 1931.

The incidence of Protozoa in domestic swine by J. E. Alicata.—Press preparations of portions of the diaphragm of 180 hogs which had been raised in California and fed on garbage, showed that 135 animals harbored *Sarcocystis miescheriana*, the incidence of infestation being 75 per cent. Fecal specimens obtained from the colon of 35 pigs which had been shipped to Benning, D. C., from National Stockyards, Ill., were examined for protozoan parasites with the following results: One specimen contained cysts indistinguishable from those of *Endamoeba histolytica*; 15 samples contained cysts of *E. polecki*; 9 samples contained cysts indistinguishable from those of *Iodamoeba butschlii*; 1 sample contained cysts indistinguishable from those of *Endolimax nana*; 12 showed cysts and trophozoites of *Balantidium* and 29 contained *Eimeria*. The oocysts of *Eimeria* showed considerable variation in size.



Paul Bartsch reported two cases of *Leishmania* at Freedman's Hospital, Washington, D. C., which apparently were of local origin. He also reported on a case of amoebiasis with extensive involvement of the liver.

The occurrence of a species of *Capillarinae* in the gastric wall of rats in the United States by J. E. Alicata and J. T. Lucker.—Female nematodes, tentatively regarded as *Hepaticola gastrica* Baylis, 1926, have been found in the mucosa of the cardiac portion of the stomach in seven out of ninety rats, *Rattus norvegicus*, which were captured in Washington, D. C. The morphology of the females and eggs agrees well with descriptions of *H. gastrica*, more particularly with that of Vogel (1929). A careful search for carcinomatous growths in the tissue was not made, but no gross abnormalities were noted. Since the publication of Baylis' description of *H. gastrica*, two proposed species of *Hepaticola*, namely *H. muris* and *H. cancerogena*, inhabiting the gastric wall of rats, have been described with special reference to pathological changes in the stomach wall attributed to their presence. Aside from *Gongylonema neoplasticum*, no nematodes have been previously reported from the gastric mucosa of rats in the United States.

L. A. Spindler presented a note on abnormalities in nodular worms of swine. (To be published elsewhere.)

The occurrence of *Euparyphium inerme* (Fuhrmann) in North America by E. W. Price.—Recently a specimen of trematode was sent to the Bureau of Animal Industry for identification by Dr. Ronald G. Law, Kirkfield, Ontario, which had been collected from a mink, *Mustela vison*. A comparison of this form with Fuhrmann's (1904) description of *Echinostomum inerme*, a species collected from *Lutra* sp. in Java, indicates that the two forms are probably identical. Some slight differences were noted but these were so slight that they appear to be insignificant.

The following note on *Stephanurus dentatus*, found among the unpublished papers of the late Dr. Brayton Howard Ransom, was read by Benjamin Schwartz.—Two hog hearts forwarded by Dr. Henry Marshall, Richmond, Va., October 19, 1915, each showed a cyst on the wall of the ventricle. In one case the cyst measured about 10 mm. in diameter, in the other 6×4 mm. The wall of the cysts was thick and fibrous; the cysts contained a small amount of pus and black pigment, also immature nematodes of the species *Stephanurus dentatus*. The worm in the larger cyst measured 14 mm. in length, in the smaller 4.5 mm. in length. These cysts somewhat resembled degenerate cysticerci. The black pigment, however, is not characteristic of tapeworm cysts.

W. H. WRIGHT, *Secretary*

## BOOK REVIEWS

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CONTRIBUIÇÃO AU ESTUDO DA BIOLOGIA DO "STRONGYLOIDES STERCORALIS." By HEITOR PRAGUER FRÓES. 122 pp. Bahia, 1930.

This investigation was undertaken to determine certain biological questions arising from the discovery of *Strongyloides stercoralis* larvae in the hemorrhagic pleural exudate and pericardial fluid of a native of Bahia. After considering the classification and life cycle of the nematode, the tactic reactions of the larvae, the mechanism by which man becomes infected and the route of migration through the body, the investigator then lists the cases in which *Strongyloides* larvae have been recorded from unusual foci in the body, namely peripheral blood, urine, sputum, liquid vomitus, and pleural and pericardial fluids. A brief section is devoted to the histopathology of the infection. It was concluded that the larvae found in the pleural and pericardial fluids were rhabditiform and did not presuppose a reinfection of the patient; that there may be either a direct or an indirect type of life cycle, depending on factors thus far unknown; that the parasitic form is hermaphroditic and not parthenogenetic; that the portal of entry into and migration through the body is analogous to that of the hookworm; that in massive infection or reinfection a severe pruritic urticaria may result; that the organism normally inhabits the small intestine, particularly the duodenum and upper jejunum, and that the eggs develop in the glands of Lieberkühn, where they embryonate and hatch and the larvae, escaping into the intestinal lumen normally pass out in the stools. On the whole the study is a valuable critique of the present status of the *Strongyloides* problem, although the author has added little original material to the subject.

LA FIÈVRE ONDULANTE. By H. VIOLE. 114 pp. Masson et Cie, Editeurs, Paris.

Until relatively recent times undulant fever has been regarded as essentially a disease of the circum-Mediterranean area. However, it has been observed most recently in distant parts of the world and has come into notice prominently in this country also. It has been strikingly and correctly designated by Professor Charles Nicolle as the disease of the future. As such and as a malady recently demonstrated in our own country, it is of special interest to students in medical zoology. The author of this little book which is an item in the series of manuals issued by the same publishers, is a member of the Higher Council of Public Hygiene in France. He has presented here an outline of the disease in brief form. Emphasis has been laid on the side of prophylaxis although the various features of the complaint are handled in systematic order.

| MAGAZIN DE PARASITOLOGIE DU MUSÉE ZOOLOGIQUE DE L'ACADÉMIE DES SCIENCES DE L'URSS. Leningrad, 1930, I. 260 pp., 22 plates. 1931. II. 325 pp. Many tables, maps, figures.

The appearance of a new journal in the field of parasitology is naturally the occasion for considering those developments which have led to its establishment and are relied upon for its support. In the Soviet Union extraordinary activity in scientific research has been seen in recent years and in no fields more worthy of note than in parasitology. This has been evidenced by the establishment of special technical schools and courses, by the organization of expeditions to study regions hitherto unexamined in this respect and by the extensive series of publications

from an increasing number of Russian investigators. An Institute of Applied Parasitology which has been established at the University of Leningrad under the leadership of Professor E. N. Pavlovsky, is one of the latest and most important of these enterprises. Several series of text books for different groups of workers have been published lately; some of them have been reviewed in the *JOURNAL*. Expeditions to investigate parasitology have been sent out into Siberian provinces and into Turkmenia, Armenia, and other parts of Asiatic Russia. The results of these and other projects not detailed here have been incorporated in an extensive literature only partially and imperfectly known in this country.

Recently the Academy of Sciences in the S. S. S. R. has established a new journal devoted specifically to the field of parasitology and has elected Professor Pavlovsky as its editor. The *Magazin de Parasitologie* has been inaugurated as an annual of which the first issue (1930) contains a dozen articles by as many investigators on parasitologic topics of wide variety in biological as well as geographic range. The text is Russian and most articles are provided with a good résumé in French or German. The second volume (1931) just received is of even greater size and equally high character. One notes with interest that most of the twenty articles included in the second volume are devoted to insects and their specific part in disease transmission. Soon the editor expects to issue the publication quarterly in the style of the *JOURNAL*, as he states in a personal letter, adding "we follow with the greatest interest the brilliant advances of experimental parasitology in the United States."

A GUIDE TO HUMAN PARASITOLOGY. By D. B. BLACKLOCK and T. SOUTHWELL. 271 pages, 2 colored plates and 122 illustrations. H. K. Lewis & Company, Ltd., London.

To a considerable series of texts in parasitology which have appeared recently the authors have added a work which has a purpose of its own. It is planned for students in special courses and for practitioners who have no laboratory at hand but who are required to make diagnoses of diseases caused by animal parasites. Long experience in teaching has shown the authors that the practitioner is distracted by the flood of names and details so as to miss the few essentials of differential diagnosis. The authors confess to unorthodox tendencies and limit their emphasis to pathogenic organisms and features useable for ready diagnosis. Such treatment is unquestionably useful in routine diagnosis, even though it breaks down in unworked regions or when new conditions are encountered. The text is well written and shows the clarity of treatment characteristic of a good teacher. The various schemes, tables, synopses, and keys, of which there is a goodly number, are admirable aids for the ordinary worker and should be highly commended. Furthermore, no one can go astray if he heeds the advice of the authors that in cases of doubt more extensive works such as are cited, should be consulted.

SEXTA REUNION DE LA SOCIEDAD ARGENTINA DE PATOLOGIA REGIONAL DEL NORTE. 880 pages, many illustrations. Imprenta de la Universidad, Buenos Aires. 1931.

Previous volumes in this series have been reviewed in earlier numbers of the *JOURNAL*. The work of this Society is outstanding in its character and merits the careful attention of parasitologists everywhere. Like its forerunners the present volume evidences the same deep interest in problems in this field and like scientific acumen in the attack on their solution. It is dedicated to commemorate the semi-centennial of the discovery of the malarial parasite by Laveran. The frontispiece is a portrait of Laveran and the opening article recounts in brief his scientific work.

In a well printed and admirably illustrated volume of nearly 900 pages the Society presents 75 papers grouped under ten sections of which the following are of particular interest to parasitologists: mycology, microbiology and experimental pathology, paludism and hematology, medical entomology, human and comparative parasitology, and natural history. The Society is performing an important function in inspiring such extensive and important work and in publishing the results in such adequate fashion. South America is almost an unworked field and offers new and serious problems to the parasitologist. It is fortunate that this work is being developed under such favorable conditions.

DIE NEMATODENFAMILIEN CUCULLANIDAE UND CAMALLANIDAE NEBST WEITEREN BEITRÄGEN ZUR KENNTNIS DER ANATOMIE UND HISTOLOGIE DER NEMATODEN. By NILS TÖRNQUIST. 441 pages, 17 plates. Wettergren & Kerber, Göteborg.

This splendid monograph is heartily welcome. No group is in greater need of thorough detailed study than the nematodes. The technical difficulties involved in handling these parasites are in large part responsible for the mass of superficial and imperfect papers that have essayed to describe and classify representatives of this group. Törnquist has taken two widely distributed families on which there exists an extensive literature and has subjected the species to careful anatomical and histological study. Every student of the field will recognize the value of the results the author has achieved. The work is well printed and finely illustrated. It will be of great service to parasitologists.

COMMON PESTS. By RENNIE W. DOANE. 384 pages, 215 illustrations. Charles C. Thomas, Springfield, Illinois.

The larger part of this book is devoted to Insect Control and the insect pests of garden, field, and home. The first section contains a concise and useful survey of parasites of man and the domestic animals. Mosquitoes, flies, other blood sucking insects, and the diseases they transmit as well as parasitic worms, are discussed together with methods of control. The book is well illustrated and is a good work for ready reference.

ARCHIVOS DO INSTITUTO BIOLOGICO DE DEFESA AGRICOLA E. ANIMAL. 320 pages, 35 illustrations. Secretaria da Agricultura, Industria E. Comercio, São Paulo, Brasil.

Volume 4 for 1931 of this important publication has just appeared. It maintains the high standing in contents and in makeup which has characterized earlier issues. One of the most important features of the present volume is a series of articles on the disease-producing organisms demonstrated in the parrots of the Amazon. The findings are of direct significance in connection with the outbreaks of psittacosis which have commanded public attention in this country especially within the year. The illustrations accompanying these papers are worthy of high commendation.

In the *Indian Medical Research Memoirs* I. M. Puri has written on the Larvae of Anopheline Mosquitoes, with full description of those of the Indian species. This is an exhaustive illustrated monograph of the species found in India. It contains a general discussion of structure and a systematic section giving

synoptic tables and details of the external features of all recorded species. Its value is evidently greatest for local workers on mosquito-borne diseases and similar problems.

*The National Medical Journal of China* has issued a special Parasitology number (October, 1931). It contains 22 papers on the clinical, experimental, histological, epidemiological, taxonomic and historical phases of the subject. In discussing the issue the editor writes: In these days of intense specialization one is apt to lose sight of the vital relationship which the science of medicine bears to general biology. The study of parasites and of the diseases they engender in man and animals helps us to keep constantly in mind this relationship. This fact alone is weighty enough to justify the issue of this special number on Parasitology. If another reason is required, it will be the unique position the subject occupies in the field of medicine. Parasitology rivals bacteriology in its influence on the development of preventive medicine and was probably solely responsible for the introduction of chemotherapy by Ehrlich.

The prevalence of wide spread and serious parasitic diseases in Africa, both among men and among other mammals, make studies from that region of particular interest. *The Seventeenth Report of the Director of Veterinary Services and Animal Industry*, Onderstepoort, Pretoria (1931) contains a long series of papers on researches in protozoal diseases, parasitology, and other allied fields. Of especial interest to parasitologists may be mentioned papers on hematozoa and on nematoda.

*The Specific Treatment of Human Schistosomiasis (Bilharziasis)* by Doctor M. B. Khalil has recently been published (Beihefte zum Archiv für Schiffs- und Tropen-Hygiene, Bd. 35, No. 2). The author gives a critical discussion of various treatments and the results obtained. An extensive bibliography is appended in each case with chemical and pharmacological data.

*The Regulation of Size as Illustrated in Unicellular Organisms* by Edward F. Adolph (C. C. Thomas, Springfield, Illinois) discusses interesting biological problems in attractive manner. Some of the organisms studied were parasites, but the data available were rather meager to justify general conclusions in this field.

The London School of Tropical Medicine has issued in its Memoir Series, *A Helminthological Survey of Southern Rhodesia*, by William K. Blackie. The study was organized to cover the indigenous natives, those of mixed stock and the migratory population as well as Europeans. It gives thus a true view of the relative importance of various helminthic diseases. Another number in this series, *Researches on Blackwater Fever in Southern Rhodesia* by G. R. Ross, represents a continuation of work done by Thompson in 1922 and 1923. The entomological section of the report is printed separately. This part deals with epidemiology on the basis of intensive studies covering several years.

Under the title of *Recherches sur les Spirochétidés dans le district de Montréal*, Doctor G. Gardner has published a booklet containing a biological study from a region as yet little known through parasitologic investigation. Unique are the author's experiments on the influence of cold on spirochaetes.



The Sociedad Argentina de Profilaxis y Patologia de la Hidatosis has been organized on the proposal of Doctors J. Arce and O. Ivanisevich. The Instituto de Clinica Quirurgica of Buenos Aires serves as headquarters and the society aims to promote scientific research on the frequency, problems and control of the disease.

In the Bibliotheka of Helminthology issued by the U. S. S. R., a recent work is a book by Doctor R. Ed. S. Schultz on *The Parasitic Worms of Rabbits and Foxes and the Diseases Produced by Them*. It is a complete and thorough presentation of the species thus far reported. Noteworthy is the amount of American literature cited under the species. Except that the text is Russian, the book might become useful here.

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#### ERRATA

In the December, 1931, issue of this journal—No. 2, Vol. 18—on page 61, in the Notes on Californian Myxosporidia by A. Pringle Jameson *Ceratomyxa taenia* should read *Ceratomyxa habena* and *Ceratomyxa venusa* should read *Ceratomyxa venusta*.

#### DINNER TO DR. ALBERT HASSALL

In honor of his seventieth birthday friends of Dr. Hassall, senior zoologist and assistant chief of the zoological division of the Bureau of Animal Industry, gave him a dinner on February 12.

Doctor Benjamin Schwartz was toastmaster and the following persons spoke on Dr. Hassall's achievements and personality: Dr. Paul Bartsch of the National Museum, Dr. N. A. Cobb of the Bureau of Plant Industry, Dr. W. W. Cort of the School of Hygiene and Public Health, Johns Hopkins University, Dr. M. C. Hall of the Bureau of Animal Industry, and Dr. U. G. Houck, Associate Chief of the Bureau of Animal Industry.

Dr. Hassall is the veteran of the Bureau, having been for 45 years in its service. The index-catalogs of which he has been co-author are regarded as among the most important reference works in the field of parasitology and as indispensable guides to the literature. The author catalog is being republished and brought up to date, and Dr. Hassall's term of service has been extended to enable him to continue this work.

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#### IN MEMORIAM

Just as this issue goes to press the sad news comes of the sudden death of Doctor Nathan A. Cobb of Washington, D. C., widely known for his remarkable studies on nematodes. He was a contributor to the first number of the Journal and has been its constant supporter. The present number can not be closed without placing on record this line of appreciation of his able efforts in this field and his personal worth.

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## THE FUTURE OF THE JOURNAL

The foundation of the JOURNAL OF PARASITOLOGY followed years of discussion and the accumulation of evidence that such a publication was demanded by the growth and importance of the new field. The first issue appeared in December, 1914. In the brief announcement with which that number opened, the purpose of the JOURNAL was stated as "the dissemination of knowledge and the encouragement of teaching and research in parasitology."

In the series of eighteen volumes, closing with the present issue, the JOURNAL has published about 500 scientific articles together with notes, society proceedings, book reviews, and records of special interest to parasitologists. Its work has been given frequent approval and increasing support. The remarkable increase in general interest, as well as in number of workers and volume of product in parasitology, is generally recognized. Some small part of this may reasonably be attributed to the opportunities offered and influence exerted by the JOURNAL.

In 1925 after extended preliminary discussion the American Society of Parasitologists was organized and the event recorded in the JOURNAL. The announcements and proceedings of the Society have appeared regularly in its pages since that date.

At the first annual meeting of the Society, held in Kansas City, a committee on publication was appointed and I was made its chairman. At that time I stated personally that I regarded the JOURNAL OF PARASITOLOGY as not in the true sense personal property but rather as held in trust for the development of the field and as such naturally transferable to the Society whenever conditions justified the change. Similar statements were made at later committee meetings and in business sessions of the Society. In 1927 a poll of the membership showed sentiment in favor of taking over the publication, but a special committee in working out the plan did not find sufficient financial support to justify assuming the responsibility.

A new survey of the situation, undertaken this spring after action by the Society at New Orleans as recorded in the JOURNAL for March, has demonstrated adequate support. The Council has approved the findings and signified its willingness to assume the responsibilities for the Society. Hence the way is clear to carry out my oft expressed intention and desire which I do hereby in transferring the JOURNAL without incumbrance or obligation to the care and control of the American Society of Parasitologists. It is understood that I will meet all obligations connected with the past and that the Society will assume all involved in publishing future numbers.

All realize at once as I do that this transfer of ownership involves no change in purpose but only assures the better realization of those ideals which started the project and have supported it through many trying experiences in these eighteen years. It has not been a one man job. If it had not been for the constant help of members of the Editorial Board and of other friends and fellow workers the results could not have been achieved. In every emergency some one has come forward to give the needed help. With a young, vigorous and growing society behind it the JOURNAL will do greater work in disseminating knowledge and developing its field.

HENRY B. WARD.

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